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Some Effects of Accumulated Radium on the Productivity of Algae

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SOME EFFECTS OF ACCUMULATED RADIUM ON THE PRODUCTIVITY OF ALGAE

by

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TABLE OF CONTENTS

LIST OF TABLES

Table		Page

LIST OF FIGURES

INTRODUCTION

Ionizing radiation from natural occurring radionuclides and cosmic rays has always been a part of man's environment, but at such low levels as to have had virtually no effect. However, with the advent of the atomic age and the advancement of nuclear technology, background activity has measurably increased all over the world. Fallout from nuclear weapons testing, wastes from nuclear reactors, and wastes from the milling of radioactive ores have all contributed to this increase. Due to this increase, many ecosystems and their individual components are now subjected to radiation levels as high as a million times natural background. What possible effects this may have has become an area of great concern and interest.

The presence of radium in many of the waters of the United States has been known for years, but in the last 15 years due to the increased demand for uranium, it has become a pollutant of considerable consequence (Coulomb, 1955; Rone, 1952; Schlundt, 1910; Scott and Barker, 1956; Scott, 1961; Tsivoglou et al., 1958). The artificial distribution of radium as a pollutant in streams seems to be unique to the uranium refining industries. Radium is a daughter product of uranium and a by-product in the processing of uranium ore (Glasstone, 1958; Tsivoglou et al., 1958). It is released into the streams as a part of the refinery waste, generally in the form of a chloride or sulfate. Examples of this type of pollution occur in the Animas, Dolores, and San Miguel Rivers of southwestern Colorado.

Surveys by the U. S. Public Health Service (Tsivoglou et al., 1958, 1959) found the dissolved radium in mill wastes ranged from 44 to 822 picocuries radium per liter, but the highest level recorded in the streams of concern never exceeded 55 picocuries radium per liter (U. S. Geological Survey, 1959). This, however, is much higher than concentrations found under natural conditions.

Since aquatic organisms may be expected to contain radioactive materials that are present in the surrounding water and sediments, the U. S. Public Health Service was interested in determining the extent various components of the aquatic biota incorporate and concentrate the radium at the levels present in the streams. To this end the U. S. Public Health Service financed a portion of this project through their National Institutes of Health contract number RH-00077. The Atomic Energy Commission supported a portion of this study on contract number AT(11-1)-1023. The Atomic Energy Commission was interested in changes in the aquatic ecosystem that might be attributed to chronic exposures to dissolved radium. The portion of this study supported by the Atomic Energy Commission was devoted to controlled laboratory experiments concerned with the effects of low level radiation from radium on the ecosystem, since determinations of the effect of low level radiation are difficult to make in the field.

This was basically a problem in radiation ecology. However, before the community dynamics could be understood, the effects of radiation upon the individuals' physiological responses to the stresses of the environment had to be evaluated. It was necessary to establish the effect of radium on the primary producers in the stream since an adverse response by an organism to a factor such as ionizing radiation might affect the entire ecosystem.

The primary or basic production of a stream depends chiefly upon the benthic algae present. These algae are the first link in the food chain and the entire pyramid of the aquatic ecosystem depends upon this primary production as the fundamental step. There is a good deal of evidence that algae tend to concentrate radium and concentration factors have been reported ranging from 2 to 1000 (Koczy, 1961; Wiesner, 1938; Brunovskii and Kunasheva, 1935; Brunovskii, 1932; Vernadskii, 1930). If the algae in these polluted streams concentrate the radium and since the food chain in the stream is so short, the entire biota is potentially a source of radiation.

The main objectives of this study were:

1. To determine relative uptake rates and concentration factors of radium by several algae exposed to different concentrations of dissolved radium for varying lengths of time.
2. To determine if exposure to different concentrations of dissolved radium for varying lengths of time affects the production of these algae.

REVIEW OF LITERATURE

Characteristics and occurrence of radium

Radium 226 is a naturally occurring radionuclide possessing a half life of 1620 years. It is a member of the Uranium-Radium Series.

On the basis of its atomic structure with two valence electrons, radium belongs to Group II-A known as the alkaline earth elements. Other members of this group are beryllium, magnesium, calcium, strontium, and barium. In their free state they are all highly metallic with a grey white luster that tarnishes rapidly in the air. Radium has an atomic number of 88 and an atomic weight of 226.05. It has a melting point of 960 C and a boiling point of 1140 C. Radium is usually prepared for commercial use in the soluble chloride form. This is also the principal manner in which soluble radium enters streams as waste from uranium ore refining. In commercial extractions of uranium ores the radium is precipitated as a relatively insoluble sulfate and then reclaimed by conversion into radium chloride (Latimer and Hildebrand, 1951). Radium chloride was the source of radium for this study.

Radium does not exist free in nature. It is widely distributed in almost all rocks but in extremely small quantities. The estimated percentage of radium in igneous rock is 10^{-12} (Latimer and Hildebrand, op cit.). The principal uranium ores contain about 3.4 x 10^{-7} grams of radium per gram of uranium. The principal ores of uranium are pitchblende or uranite, and carnotite (Holmes, 1949). Extensive deposits of carnotite are found in Colorado, Utah, and Arizona.

In order to understand the role of radium in the ecology of the

Table 1. The Uranium-Radium Series

Radioelement	An isotope of	Symbol	Radiation	Half-life
Uranium I	Uranium	U-238	Alpha	4.5×10^9 yrs.
Uranium X_1	Thorium	Th-234	Beta	24.1 days
Uranium X_2	Protactinium	Pa-234	Beta	1.18 min.
Uranium II	Uranium	U-234	Alpha	2.7×10^5 yrs.
Ionium	Thorium	Th-230	Alpha	8.2×10^4 yrs.
Radium	Radium	Ra-226	Alpha	1.6×10^3 yrs.
Radon	Radon	Rn-222	Alpha	3.82 days
Radium A	Polonium	Po-218	Alpha	3.05 min.
Radium B	Lead	Pb-214	Beta	26.8 min.
Radium C[a]	Bismuth	Bi-214	Alpha,Beta	19.7 min.
Radium C'	Polonium	Po-214	Alpha	1.5×10^{-4} sec.
Radium C' '	Thallium	Tl-210	Beta	1.3 min.
Radium D	Lead	Pb-210	Beta	22.2 yrs.
Radium E	Bismuth	Bi-210	Beta	4.97 days
Radium F	Polonium	Po-210	Alpha	139 days
Radium G	Lead	Pb-206		Stable

[a]Radium C may disintegrate either by emitting a beta particle to form Radium C' or by emitting an alpha particle to form Radium C' '. Modified from Young, 1957, p. 86.

aquatic community it is first necessary to understand its geochemical behavior. This cannot be separated from that of the other long lived radionuclides such as uranium and thorium. In the course of the decay of an atom of uranium it will twice be an isotope of thorium and an atom of thorium will twice be an isotope of radium (Hutchinson, 1957). The average values of the measurments of these elements in water agree well with their general solubility properties (Koczy, 1961). Koczy states that as long as uranium is in an oxidizing environment it is quite soluble as a hexavalent form in a uranyl complex. On the other hand, uranium in water with a low redox potential is reduced to a tetravalent state in which it has a high affinity for organic matter. Uranium is usually found in high concentrations in well-aerated water due to its high solubility as uranium tetracarbonate. Thorium, the daughter of uranium, is easily precipitated or adsorbed and is also quite insoluble. Therefore, according to Koczy (1961), it is generally in low concentrations in natural waters. Radium in the form of a bicarbonate or chloride is water soluble but it is precipitated when in the form of a sulfate. Because of this and the insolubility of its predecessor, thorium, radium is generally found in lower concentrations than would be expected when compared to concentrations of uranium (Koczy, 1961; Arnold and Martell, 1959).

A radionuclide such as uranium, which has a very long half-life, will decay with the production of daughter products. These daughter elements will continue to accumulate until they reach a state of radioactive equilibrium at which point their decay rates just equal the rates at which they are produced. The ratio of uranium to radium at such an equilibrium is 3 x 10^6 to 1 (Hutchinson, 1957). These particular nuclides will not reach this state of radioactive equilibrium in natural waters. This is due to the ever-changing state of any given water and the complexity of chemical behavior (Picciotto, 1961; Klement, 1962).

There have been many analyses of waters for both uranium and radium. Most of the early measurements of the radioactivity of fresh waters were accomplished in a search for springs and wells of high activity for health spas. There is some question, however, as to the validity of some of the earlier results (Koczy, 1961; Hutchinson, 1957). Another source of confusion in making comparisons of the available data is the wide variation of units in which the radioactivity was expressed.[1] Even those results reported in similar terms may have considerable differences due to different analytical procedures.

Most of the early measurements of radium in fresh waters indicated the concentrations were about 0.5 picograms of radium per liter[2] (Korarik and McKeehan, 1925; Meyer and Scheidler, 1927). Concentrations in river waters usually range from about 0.01 to 0.1 pg Ra/l though higher and lower concentrations have been reported (Klement, 1962). According to Federov and Baranov (1956), the fresh waters in the U.S.S.R. normally contain about 1 pg Ra/l. The analysis of Vienna tap water was at the lower end of the expected range for dissolved radium in fresh water. It was reported to contain 6 x 10^{-2} pg Ra/l (Pertz, 1937). Brunovskii

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[1]For the sake of clarification in this report, the units have been converted as nearly as possible to the same base.

[2]The unit prefix pico (p) will be used in the text where applicable. The conversion units are as follows:
1 μg/g = 1 ppm = 10^{-4}% by weight; 1 pc = 1 $\mu\mu$c = 10^{-12} c.
1 g RA = 1 curie of activity = 3.7 x 10^{10} disintegrations per second.

and Kunasheva (1935) found the pond at the Peterhof Orangery near Leningrad contained concentrations of 16×10^{-2} to 51×10^{-2} pg Ra/l. River water was reported to range from 0.2 to 4 pg Ra/l in the U.S.S.R. (Belousova, 1961), from 0.07 to 0.84 pg Ra/l in Germany (Muth et al., 1960), and an average of 0.1 pg Ra/l was reported for surface waters of the U. S. (Lucas and Ilcewicz, 1958). Hursh (1957) claimed the western part of the U. S. had natural waters with concentrations ranging from 0.04 to 440 pg Ra/l.

Artificial distribution of radium has added to the amounts in some streams. It has been estimated that 10 curies of radium per day were dumped into streams of the western U. S. by uranium refining plants (Eisenbud, 1963). Tsivoglou et al. (1959, 1960) reported such a situation in the Animas River in Colorado and New Mexico. A mill processing uranium and vanadium ores dumped the effluent, containing radium, into the river. Samples of river water averaged 12.6 pg Ra/l near the mill and 2.9 pg Ra/l 60 miles away. The tributaries contained from 0.3 to 0.6 pg Ra/l. Gahr (1959) reported the Colorado River below Grand Junction, Colorado, had 39 pg Ra/l compared to 0.3 pg Ra/l above and the San Miguel River below Uravan, Colorado, contained 86 pg Ra/l to 3.9 pg Ra/l above.

Documentation of measurements of concentrations of other radionuclides of long decay series are far more scarce than those on radium. Thorium has not been measured at all in river waters. Koczy (1961) stated he and Picciotto failed in their attempt to determine the thorium 230 and thorium 232 concentrations in fresh water; however, he arrived at a concentration of 4×10^{4} pg Th/l by an indirect method.

Uranium has been investigated extensively and a wide range of concentration values has been reported. Hoffman (1942) published determinations of Austrian river waters that ranged from a concentration of 16×10^4 pg U/l to 47×10^4 pg U/l. In the U.S.S.R., it was reported that lakes had an average of 8×10^6 pg U/l and river water averaged 6×10^5 pg U/l (Tokarev and Shcherbakov, 1956). In the U.S., Judson and Osmond (1955) measured concentrations of from 13×10^4 to 35×10^5 pg U/l in surface waters from Wisconsin, Illinois, and Texas. The following table summarizes the most reputable measurements of radionuclides in river water.

Table 2. Maximum and average values of U, Th 232, and Ra 226 in river water[a]

	Average	Maximum	Equilibrium value
Uranium	5×10^5 pg/l	2×10^8 pg/l	
Thorium 232	4×10^4 pg/l	2×10^5 pg/l	
Radium 226	3×10^{-2} pg/l	10^2 pg/l	18×10^{-2} pg/l

[a]From Koczy, 1961.

There are several recent reviews on the natural radionuclides in the marine environment (Suess, 1958; Picciotto, 1961; Koczy, 1961; Klement, 1962; Koczy and Rosholt, 1962).

Radionuclides are found in the ocean in different concentrations than in fresh water. Unless the particular area of the ocean into which uranium enters is in the state of a reducing environment, the uranium merely joins that already present and increases the uranium content of the water (Koczy, 1961). Concentrations of uranium reported in the ocean, regardless of the region of the sample, remain fairly constant. It ranges between 3 and 3.4 x 10^6 pg U/l (Smith and Grimaldi, 1954; Steward and Bentley, 1954; Koczy et al., 1957a). Foyn, Karlik, Petterson, and Rona (1939) give lower estimates. They claim sea water varies from about 1.1 to 1.8 x 10^6 pg U/l with the best average value being 1.3 x 10^6 pg U/l.

Concentrations of radium in the ocean water are lower than those in fresh water. Koczy (1958) gave the range of concentrations as 2 x 10^{-1} to 19 x 10^{-1} pg Ra/l. Hutchinson (1957) claimed the best values lie between 8 x 10^{-1} and 9 x 10^{-1}pg Ra/l. This concentration of radium would be in equilibrium with approximately 2.5 x 10^5 pg U/l. Since this value is lower than the lowest uranium determinations, it is obvious that radium is continually being lost from the ocean. This is accomplished through the precipitation of its precursor thorium 230 (Hutchinson, 1957; Arnold and Martel, 1959; Koczy, 1961). Thorium precipitates so completely that no determinations have been made of the concentration of thorium in ocean water. Koczy (1961) gave a value he arrived at by deduction of 2 x 10^2 pg Th/l. The following table (Table 3) gives some average values for concentrations of these radionuclides in the ocean.

Comparing the tables on average concentrations of radionuclides in fresh and sea water, we can readily note the differences. These are due to the chemical differences in the water and the solubility properties of the radionuclides. Because the solubilities of nuclides from

Table 3. Estimated average concentrations of radionuclides in sea water[a]

Nuclide	Concentration
Uranium 238	3×10^{6} pg/1
Thorium 232	2×10^{4} pg/1
Thorium 230	3×10^{-1} pg/1
Radium 226	1×10^{-1} pg/1

[a]Abbreviated and modified from Klement, 1962.

the natural radioactive series are affected by changes in alkalinity and carbonates, uranium is more soluble in the ocean than in fresh water; thorium is less soluble in the ocean than in fresh water; and radium is more soluble in the ocean than in fresh water.

Incorporation of radionuclides by algae

There are many data on the uptake and concentration of radionuclides by plants and animals (Vernadskii et al., 1937; Scott, 1955; Foster and Davis, 1955; Borough et al., 1957; Krumholz et al., 1957; Krumholz and Foster, 1957; Tsivoglou et al., 1958, 1959; Klement, 1962; Gilvea, 1963). These studies were, for the most part, concerned with organisms living in waters used for nuclear tests, reactor wastes, or waste disposal from industrial operations. The possible radiation hazard from contaminated food prompted most of the studies. Information on the uptake of natural occurring radionuclides is relatively scarce.

Information on uranium uptake by aquatic plants is practically nil. Hoffman (1942) reported 9×10^{6} pg U/g ash weight of green algae.

The algae were found in water containing 4×10^3 pg U/g so, following the assumptions of Hutchinson (1957) that ash weight is 5 percent of dry weight and dry weight is about 10 percent of wet weight, we arrive at a concentration factor of about 10. Plant material generally ranges between 0.1 and 34×10^6 pg U/g. In reducing environments, decaying organic matter and sediments containing a high percentage of organic matter may have extremely high uranium contents. This, however, appears to be a secondary process (Koczy, 1961).

Data indicate that radium is concentrated much more than uranium or thorium by various organisms. Weisner (1938) reported marine algae containing 9.2×10^{-2} pg Ra/g dry weight, Cladophora with 14×10^{-2} pg Ra/g dry weight, and Zygnema with 17.3 pg Ra/g dry weight. Duckweed, Lemna, was found to concentrate radium 100 times over the amount in the water. Values have been reported from 1×10^{-2} to 13×10^{-2} pg Ra/g (Vernadskii, 1930; Brunovskii, 1932; Brunovskii and Kunsheva, 1930, 1935). They report a similar concentration factor for both radium and mesothorium 1. The plant would be expected to treat them similarly since MsTh 1 is an isotope of radium. Radium accumulation by plankton was reported as about 5×10^{-3} pg Ra/g or about a 10-fold concentration over sea water (Klement, 1962). In 1958, Tsivoglou et al., found up to 390 pg Ra/g ash weight of algae and in 1959 up to 880 pg Ra/g ash weight of algae in streams containing uranium ore milling wastes. The concentrations are highest just below the mill and decreased with dilution and distance from the mill.

Mechanisms of radionuclide uptake

The mechanisms of radionuclide uptake are still vague but in general may be explained by one or more of three major processes: adsorption to exposed area, absorption into tissues, or through metabolic processes (Krumholz, 1956; Davis and Foster, 1958). These were somewhat expanded upon by Laties (1959) who enumerated six processes involved in uptake and loss of ions. He listed as possible mechanisms: adsorption, adsorption-exchange (ionic exchange), diffusion, metabolic uptake, entry into free space, and leakage.

It has been demonstrated that all forms of life have basically the same metabolism at the cellular level; however, the metabolism of various radionuclides and the importance of the different means of uptake vary greatly between different species and environments (Davis and Foster, 1958). Though it has not been shown that a metal heavier than molybdenum is necessary for any metabolic process (Krumholz, 1957) radium appears to react similarly to calcium in most instances of metabolism (Eisenbud, 1963; Stover, Atherton and Arnold, 1957).

Adsorption and absorption seem to be the primary factors in the uptake of radioelements by algae. Surface adsorption of various radioelements has been observed by many authors. Spooner (1949) observed adsorption in experiments using Sr 90 and Y 90 with various seaweeds, Foster and Davis (1955) with plankton in reactor wastes, Rice (1956) and Rice and Willis (1959) in marine phytoplankton using Ce 144 and Sr 90, Chipman et al. (1958) and Gutknecht (1961) using Zn 65 with marine algae. These adsorbed radioisotopes may be more or less firmly bound or may be readily washed off (Eppley, 1962). Absorption with different radioisotopes was observed by Whittaker (1953) with P 32, Rice (1956) with Sr 90, Bachmann and Odum (1960) with Zn 65, and Williams (1960) with Cs 137. The extent of accumulation is further complicated in those algae that have calcareous skeletons into which some radioelements may be incorporated such as Sr 90 in $SrCO_3$ (Bowen, 1956).

Algae and ionizing radiation

Algae appear to be among the organisms most resistant to ionizing radiation. The LD-50 for algae is measured in thousands of roentgens and there is a time lapse of approximately a month before the results appear (Crowther, 1926; Bonham et al., 1947; Bonham and Palumbo, 1951). This figure is extremely difficult to evaluate because, in algae, the number of cells surviving a given dose is obviously a function of the initial population. LD-50 also has no comparable meaning for different organisms according to Godward (1962). This is due to the different criteria used for death and the different times at which the LD-50's are assessed. For example, various sources have determined LD-50's at 30 days for mammals, 10 to 12 days for *Chlorella*, and a "few" days for *Chlamydomonas*.

Photosynthesis and respiration have been used by many researchers to measure effects of ionizing radiation on plants. Stoklass,[1] Hruban, and Penkava (1930) observed that gamma radiation increased the rate of photosynthesis but retarded respiration. Alpha radiation inhibited photosynthesis and beta radiation inhibited it at first but after long exposure started to stimulate it. Nisima,[1] Nakamura, and Nakayama (1940) used neutron irradiation and in 3 hours found a 50 percent reduction in the

[1]Taken from Rabinowitch (1945). Photosynthesis and related processes. Vol. I. Chemistry of Photosynthesis, Chemosynthesis, and related processes, *in vitro* and *in vivo*. 599 pp. Interscience Publishers, Inc., New York, N.Y.

photosynthesis of Chlorella ellipsoidea. They found no effect on the photosynthesis of Scenedesmus nanus after 10 minutes of irradiation, a slight increase after 30 minutes, and a 37 percent reduction after 12 hours irradiation. During this time, respiration remained unaffected and remained so after 3 hours of irradiation. Henrici[1] (1921) found photosynthesis increased as much as four times in air that had been ionized by radioactive materials. Stiles and Leach (1952) reported respiration increased under the same conditions. Zill and Tolbert (1958) found gamma rays depressed carbon dioxide uptake at doses that did not affect oxygen evolution. The CO_2 fixation declined exponentially with increasing dose. The effect of 253.7 mμ photons on Chlorella pyrenoidosa was to slightly inhibit photosynthesis, greatly inhibit exogenous respiration and there was no effect on endogenous respiration (Redford and Myers, 1951). Gager (1936) reported the primary reducing process in respiration was increased by beta radiations from radium. The alpha rays from radium stimulated the oxidation products which split into carbon dioxide and hydrogen. He also reached the conclusion that the alpha rays increased all processes depending upon enzymes if hydrogen was present in sufficient amounts.

Other parameters including growth, chlorophyll formation, and various aspects of metabolism have been measured in an attempt to determine the effects of ionizing radiation.

Reported effects of ionizing radiation on the growth of plants vary. Growth rate of Scenedesmus crassus exposed to 5,000 roentgens of X-rays increased. At doses of from 25,000 to 35,000 roentgens, growth

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[1]From Rabinowitch (1945).

continued for some days and then ceased (Gilet and Ozanda, 1960). Alexander (1950) reported that fertilizers containing radioactive materials such as actinium, radium bromide, and uranyl nitrate, showed no significant effects, either beneficial or harmful, on growth of plants. Irradiation of seeds with 3,000 r. of X-rays showed no significant effects on plant growth although there appeared to be a slight stimulation (Sax, 1955). Growth of wheat seedlings was depressed by 10,000 roentgens of gamma radiation (Strazhevskaya, 1960). Porter and Knauss (1954) stated that growth of Chlorella, cultured in beta emitting solutions of $H^3 2O$, P 32, Sr 90 - Y 90, and S 35, decreased in proportion to the dose of radiation received. Numerous early experiments showed all aspects of growth such as nuclear and cell division, fruiting and flowering, photosynthesis and respiration, were stimulated by weak and retarded by strong irradiation from radium (Gager, 1936).

Another criterion for measuring the effect or ionizing radiation on algae is to determine the effect on algal production. An estimate of algal production can be obtained from measurements of the major constituents of the algae. That is, besides the pigments, the carbohydrates, lipids, and proteins (Strickland, 1960). Changes in the composition of the constituents of the cell may reflect either external changes of the environment or changes in the metabolism (Fogg, 1953).

Radiation may affect either the protein or lipids of an organism. Kuzin (1956), in his review, gave examples of both. He stated there seemed to be little effect on lipids unless the dose of radiation is quite high. The main effect in lipids appeared to be oxidative changes of some of the sterols, and the amount of oxidation seemed to be proportional to the dosage. Though most of the lipids are used as storage products, any oxidative changes in those of the lipid-protein complex of semipermeable membranes or in the chlorophyll-protein-lipid complex used for photochemical reactions could greatly affect the organism. Fernau[2] and Pauli (1915, 1929) and Arnow[2] (1935) found denaturation of protein by both radium salt and alpha particles. The amount of either lipid or protein present may be affected due to inactivation of enzymes by radiation.

Inhibition of enzyme synthesis was postulated as the possible cause for the decline of chlorophyll formation 10 hours after irradiation by gamma rays with a dose of 150,000 roentgens (Gailey and Tolbert, 1958). Spikes (1962) and Hall and VanNorman (1963) found destruction of _in vitro_ chlorophyll to be nearly linear from 0 to 32,000 roentgens of gamma irradiation.

Results from work with ionizing radiation are often in conflict due to variation in irradiation techniques or measurements of dosage. Because of this, conflicting results have been reported; therefore, no general statement can be made as to the effects of ionizing radiation on algae. Although many of the preceding works were not concerned with the effects of radium _per se_, they served as guideposts to establish the parameters of this study.

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[2]From Kuzin (1956).

MATERIALS AND METHODS

Physical setup and procedure

The ideal situation for work with benthic stream algae would be to sample under natural conditions. Due to lack of control over factors such as velocity, flow, water temperature, and concentration of pollutant, it was necessary to attempt to simulate a natural environment.

The basic unit used to simulate a natural environment was a 12-liter macrocosm consisting of a plexiglass cylinder in a closed circuit with a pump and reservoir (Figure 1). For uptake and concentration experiments the macrocosms were run in this manner. For experiments where oxygen production was used as the index of productivity, a series of clamps permitted by-passing the reservoir. This created an air-tight system with no contact between the water and the atmosphere. The tubes, hoses, reservoirs, and pumps were all color coded for identification.

In an attempt to obtain a well distributed flow, a perforated plastic plate was placed in the intake end of the tube. A dye was injected and the flow was estimated to be 1 foot per second. Ten of these tubes were placed in a wooden tank and their pumps and reservoirs in an adjoining metal trough. The tubes were placed on racks that held them just below the surface of the water and allowed the water to flow around them. Water from the river was pumped into the tank to maintain the temperature near that from which the algae were taken. Constant check

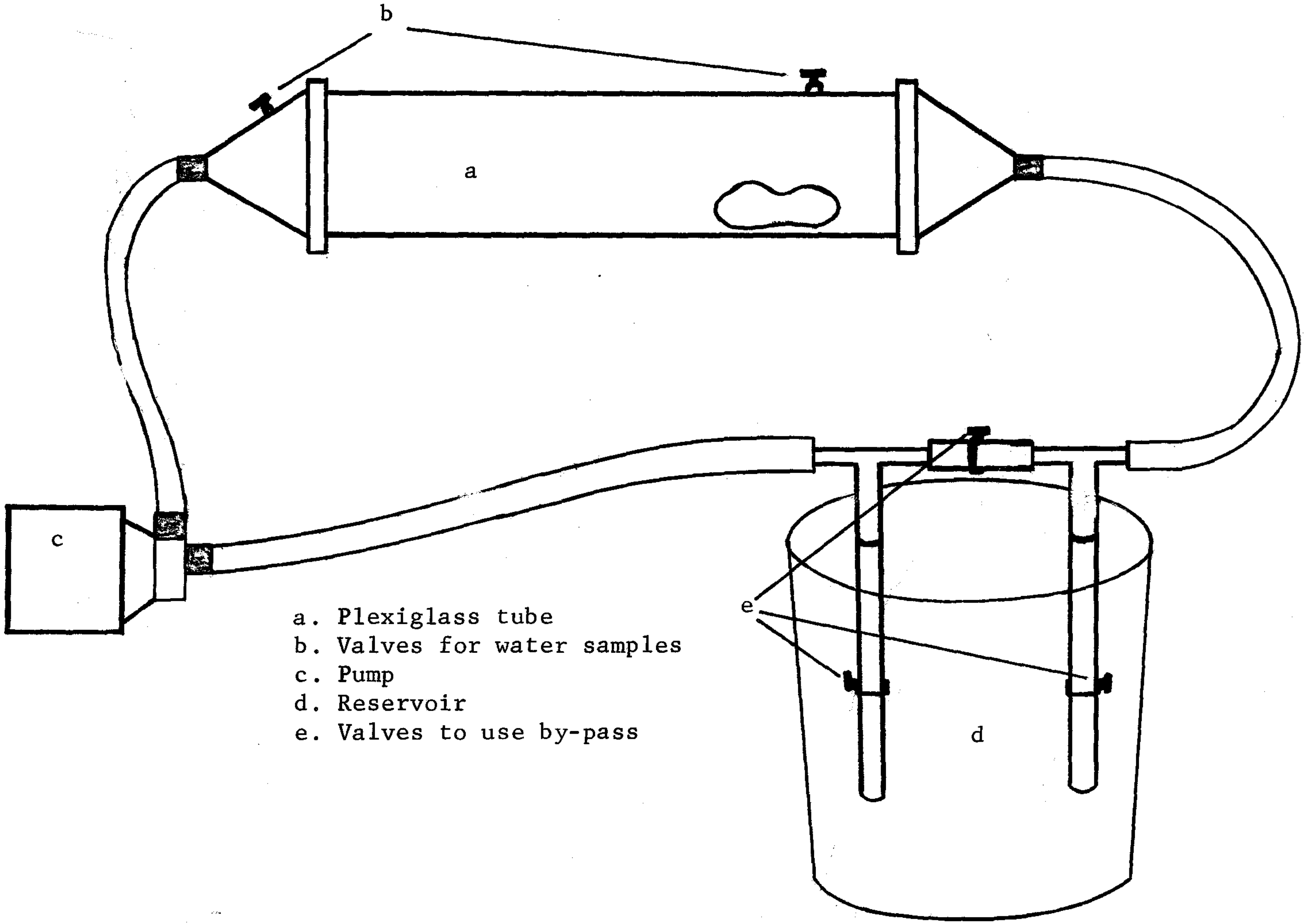

Figure 1. Experimental macrcosm used in radium uptake and oxygen production experiments with filamentous algae

of temperatures was kept with a Foxboro recording thermometer. Natural sunlight was used and to determine any great differences in the amount of light between runs, an Esterline-Angus recording milliammeter was connected to two silicon solar cell modules, SD2-1020B, from the International Rectifier Company. These photoelectric cells were mounted in a series with one of them blacked out to act as a temperature compensator.

Vaucheria and *Cladophora* were the two genera of algae used in this experimental system. These two algae were locally abundant at certain times of the year. The algae used were transplanted as excised communities from rocks in the river to small rocks in the tubes. Water from the Logan River, Utah, where the algae were gathered, was used as the culture medium to which the radium was added in the form of radium chloride.

Experiments used to determine radium uptake and concentration factors had concentrations of 0, 5,000, 10,000, 20,000, and 40,000 pc Ra/l and concentrations of 0, 1, 5, 25, and 125 pc Ra/l. After 1/2, 1, 2, and 4 days the macrocosms were disassembled, the algae were removed from the tubes, and gross alpha determinations were made.

In the measurement of oxygen production, determinations of respiration, net oxygen production, and gross oxygen production were made. The concentrations of radium used and lengths of exposure were the same as the uptake experiment using the lower radium concentrations. At each time period, a sample of water was taken to determine the dissolved oxygen content. A heavy sheet of black plastic was then placed over the tanks to exclude all light and the by-pass clamps were adjusted so that the macrocosm was an air-tight system. After two hours, another water sample was taken, the dissolved oxygen was determined and respiration calculated. The black plastic was removed, the system made air-tight again, and allowed to run another two hours. Dissolved oxygen content of the next water sample determined the net oxygen production. After the oxygen samples were taken the alga was removed from each tube and oven dried to constant weight. The final entry was made as ppm dissolved oxygen produced or consumed per gram dry weight of algae.

At the termination of an experiment the water from the macrocosms was poured into large galvanized containers lined with heavy plastic sheets. The water was allowed to evaporate and the plastic sheets were removed for burial. Each experimental unit was filled with a solution of detergent and EDTA salts (ethylenediaminetetracetate) and run for at least 24 hours. After this they were washed for 24 hours in a fresh solution. They were then rinsed in fresh water for another 24 hours. In an attempt to minimize residual effects after washing, the same macrocosms contained the same concentration of radium in each run although they still were placed randomly.

The basic unit for the experiment with a unicellular alga, Chlorella, was a 1-gallon jar containing 2 liters of Knop's culture medium. The medium was aerated and a piece of clear plastic (Saran Wrap) was secured over the top of each bottle to prevent excess evaporation. Five of these bottles were placed in the bottom of each 25-gallon fiberglass aquarium. There were 12 of these aquaria in all. Each aquarium contained enough water to surround the jars in it. All the aquaria were set in a large controlled temperature water bath where the water was circulated by a water pump to maintain even temperature throughout.

Heat transfer through the aquaria and water was sufficient to maintain a constant water temperature in the jars. The temperature was checked by an immersed maximum-minimum thermometer and a pocket mercury thermometer and stayed at 75 $\pm$ 2 F.

The Chlorella used was obtained as a pure culture from Turtox General Biological Supply House and cultured in Turtox prepared medium. The algae were concentrated by centrifuging and equal aliquots of the concentrated algae were placed in each gallon jar. The culture medium in the jars was Knop's solution as recommended in the Turtox Service Leaflet No. 6. It was assumed that equal amounts of Chlorella were placed in each jar when determinations of the dry weights of 15 samples were found to have coefficient of variation of only 4.5 percent (Table 51, Appendix B).

After the algae were added to the jars, five concentrations of radium were assigned randomly to jars in each aquarium. The concentrations of radium used were 0, 6, 30, 150, and 750 picocuries per liter. The jars were then color coded. The four sampling times of 10, 20, 30, and 40 days were assigned randomly to the aquaria.

A simulated 18-hour day and a 6-hour night cycle was created. An automatic time switch controlled eight 150W, 120V Spot Bulbs hung 9 feet above the aquaria. The light in each aquarium was approximately 150 foot-candles as measured by a Weston 756 illumination meter, and all jars received the same amount of light.

At the end of each time period three aquaria, each containing all five concentrations of radium, were sampled. The seven parameters measured on each replication were: dry weight, nitrogen content, lipid[3] content,

[3]Actually the ether-alcohol soluble constituents of the algae, but for the sake of convenience will simply be called lipids.

chlorophyll content, radium uptake, oxygen production, and carbon 14 uptake. Samples were taken by filtering through a type HA Millipore filter with a porosity of 0.45μ.

At the end of each sampling period, the algae were removed from the jars, the filtered water was poured into a washtub on a hot plate and evaporated. The concentrated radium residue remained in the tube. The jars were thoroughly washed in hot water, detergent, and EDTA, then rinsed several times. The air stones were also rinsed and flushed in fresh water for several hours. The water bath and aquaria were then washed and rinsed.

The experiment to determine the effects of radium on the uptake of carbon 14 by two genera of algae was set up somewhat like the *Chlorella* experiment. *Vaucheria* and *Cladophora*, two genera of filamentous algae, were cultured in gallon jars. These were placed in a 52 F water bath under 300 foot-candles of fluorescent light. Each jar contained 2 liters of tap water and one of five concentrations of radium: 0, 50, 150, 450, and 1,350 pc/l. Determinations of the carbon 14 uptake were made at the end of teach of four time periods: 1/2, 1, 2, and 4 days. Algae were taken from each concentration of radium at the end of a sampling period and five small pieces of algae from each concentration were placed in individual bottles. The five small pieces of algae from each treatment were placed in four light and one dark bottles. Each bottle had 0.22 μc of carbon 14 added in the form of sodium carbonate. An automatic syringe (Cornwall No. 1251BD) was used to facilitate rapid injection without danger of contamination. The bottles were then placed randomly in the water bath and left for two hours. At the end of this time they were assayed for carbon 14 uptake.

Since one of the original hypotheses for this study was that radium 226 might be passed up the food chain, a simple experiment was set up to see if a gross picture of this could be obtained. Since it is known that carbon 14 is actually incorporated into the cellular constituents of the algae, a comparison of the passage of carbon 14 and radium 226 up food chains was made using a food chain beginning with carbon 14 labeled algae and a food chain beginning with radium 226 labeled algae.

A pure culture of *Chlamydomonas* obtained from the Turtox General Biological Supply House was used as the base of the food chain. The second link was a population of mixed zooplankters. These were gathered with a coarse mesh plankton net. The final level was the mosquito fish, *Gambusia affinis*.

Chlamydomonas was cultured for three days in Turtox prepared culture medium containing either carbon 14 or radium 226. The algae was then concentrated so aliquots could be taken for the experiment. The zooplankters were also concentrated.

The basic experimental units were 100 ml disposable plastic beakers, containing 80 ml of water. There were four replications of these units for algae; zooplankton; fish; algae and zooplankton; algae and fish; zooplankton and fish; algae, fish, and zooplankton. Amounts used were 5 ml of the concentrated labeled algae, 5 ml of the concentrated zooplankton and two fish. The duration of the experiments was 2 days.

Experimental design

The uptake and concentration experiment using the higher concentrations of radium was designed as a completely randomized 4 x 5 factorial.

Four replications of Vaucheria were subjected to four lengths of exposure: 1/2, 1, 2, and 4 days; and five concentrations of radium: 0, 5,000, 10,000, 20,000, and 40,000 picocuries per liter.

The second experiment was designed to test generic differences in uptake and concentration and the effects of different concentrations of radium and lengths of exposure on productivity. It was a split-plot design with two genera of algae, Vaucheria and Cladophora, as the main plots. Within each plot were six replications of completely randomized 4 x 5 factorial consisting of four lengths of exposure; 1/2, 1, 2, and 4 days; and five concentrations of radium: 0, 1, 5, 20, and 125 picocuries per liter.

The third experiment utilized Chlorella. This experiment was designed to test effects of the concentration of radium and lengths of exposure on radium uptake and productivity of a unicellular alga. This experiment was a randomized complete block design. There were three replications of a 4 x 5 factorial with four lengths of exposure: 10, 20, 30, and 40 days and five concentrations of radium: 0, 6, 30, 150, and 750 picocuries per liter. Each 25-gallon aquarium served as a statistical block.

In this experiment each of the seven measurements taken was analyzed separately for effects due to concentration of radium and length of exposure. Next the values or coefficients of linear correlation were determined for all parameters following the procedure of Ostle (1956).

The experiment testing the generic differences of algae on carbon 14 uptake and the effects of different concentrations of radium and lengths of exposure was designed as a split-plot design with the two genera of algae being the whole plots. Within each whole plot were four replications of a completely randomized 4 x 5 factorial consisting of four lengths of exposure: 1/2, 1, 2, and 4 days; and five concentrations of radium: 0, 50, 150, 450, and 1,350 pc/l.

The food chain experiment was not designed for statistical analysis. Though it contained controls and had replications, only the mean values were used.

Procedures with filamentous algae

Gross alpha determinations. Radium analysis is a lengthy procedure, so gross alpha measurements were used as an index to the uptake and concentration of radium (Rushing, 1960; Tsivoglou, 1961). In this instance gross alpha measurements were measurements of radium content since radium 226 and its daughter products were the only sources of alpha radiation found in the water. The analysis followed the dry ash method of gross alpha determination as described by Tsivoglou (1961). The procedures were slightly modified to fit the situation and are as follows:

1. Dry algae for 24 hours at 90-100 C.
2. Weight and record dry weights.
3. Place in tared crucibles and ignite at 600 C for 12-15 hours.
4. Determine ash weight.
5. Grind ash to fine powder in mullite mortar.
6. Weigh ash onto stainless steel planchets. Divide into portions of approximately 50, 100, and 250 mg.

7. Add a small amount of reagent grade acetone to planchet to aid in the even distribution of the ash by gentle swirling. Add one or two drops (.05-.10 ml) of acetone-lucite solution to tack down the solids. One drop of acetone-lucite solution contains approximately 0.25 mg of lucite.
8. Redry planchets for 5 hours at 90-100 C. and at least 12 hours before counting.
9. Weigh planchets once more to determine no more than 0.5 mg of lucite has been added.
10. Count two successive counts in an internal proportional counter. If there are 10 or more cpm, count only 10 minutes; lower count rates are counted for 1/2 or 1 hour.

Before the planchets were used, they were treated as follows:

1. Rinse in acetone.
2. Mark on the bottom with a wax crayon for identification.
3. Place in furnace at 600 C for 30-60 seconds.
4. Remove loose crayon residue.
5. Weigh planchets.

Counts of the three different weights of ash for each sample were used in the determinations of self-absorption. At first the method of graphical analysis described by Tsivoglou (1961) was used to establish R_{oo} (count rate at infinite thickness), C (absorption constant), and r_o (count rate at zero self-absorption). An example of this method is shown in Appendix B. Though satisfactory, the procedures involved proved to be too time consuming with the hundreds of determinations involved. To alleviate this problem, Neuhold (1963) developed a computer program for gross radioactivity analysis based on Tsivoglou's method. This program was used on the IBM 1620 to obtain the final values.

Dissolved oxygen. The dissolved oxygen content of the water was determined by the sodium azide modification of the Winkler method up to the point of titration. The samples were then read on a Beckman Model B spectrophotometer. A standard absorption curve for the spectrophotometer was established and plotted so that oxygen concentration in parts per million could be determined from the optical density of the solution (Figure 31, Appendix B). In practice two determinations were made of each replication and the means were entered as the dissolved oxygen concentration.

Weight of algae. Dry weights of the filamentous algae were taken after oven drying at 90-100 C for at least 24 hours.

Carbon 14 uptake. At the end of the incubation period, 5 ml of a 4:1 solution of concentrated acetic acid and concentrated hydrochloric acid was put in each bottle with an automatic pipette to kill the algae. The algae were then filtered out and washed three times with water to remove any excess carbon 14. They were placed on previously weighed planchets and oven dried to a constant weight. The planchets were weighed again to determine the dry weight of algae. After this the algae were ground into a fine powder and spread evenly on the planchets with the aid of a small volume of reagent grade acetone. They were secured by the addition of one or two drops of acetone-lucite solution. Samples were redried and then counted in an internal proportional counter. Small pieces of algae were taken so no large effects appeared from self-absorption and no corrections were made for it. Results were tabulated as counts per minute per gram dry weight algae. Dark bottle counts were subtracted from the four replications of light bottles to get values used in analysis.

Procedures with unicellular algae

Oxygen production, carbon 14 uptake, and radium uptake of unicellular algae. For these three determinations, six 50 ml samples were taken. They were placed in clear, glass stoppered bottles. Three bottles were used for oxygen determinations and the other three were used for carbon 14 uptake and radium uptake. From each group of three samples, one was blacked out with aluminum foil and used as a dark bottle control in the experiments. The bottles to be used for oxygen production measurements were then placed back under the light for two hours. After that, dissolved oxygen was determined as described before. The remaining bottles were each injected with 3.4 μc of carbon 14, then after two hours exposure to light they were filtered. The algae were washed with 5 ml of 4:1 concentrated acetic acid and concentrated hydrochloric acid to kill them and to prevent further respiration. These filters were glued to planchets and dried. Each filter was counted in an internal proportional counter for both beta and alpha radiation. Because there was so little algae there was no attempt made to correct for self-absorption. In both instances, final results were given as counts per minute per gram dry weight of *Chlorella*.

The analyses of the planchets from the food chain experiments were carried on as above. No attempt was made to correct for self-absorption and the final results were expressed as counts per minutes per trophic level.

Dry weight determination. The quantities of Chlorella used were insufficient to be weighed on the available balances, so a method of determining weights from optical density readings was used. A pure culture of Chlorella was found to have an absorption peak at 644 mμ . Aliquots of 5 ml each were taken from a concentrated Chlorella culture and read on a spectrophotometer. The algae were filtered on tared Millipore filters, oven dried, and weighed. The original solution was then diluted by a known factor and the procedure repeated until a point was reached where there were not enough algae to weigh. A standard curve for dry weight of Chlorella was determined by plotting the grams dry weight per 5 ml of Chlorella against the optical density of the solution (Figure 32, Appendix B). Each replication in the Chlorella experiment had a 200 ml sample taken from it and filtered. The filtered algae were washed off, diluted to 10 ml, and resuspended. Three readings were taken on the spectrophotometer and the mean value was used to determine the grams dry weight of algae per milliliter.

Nitrogen content. To determine the total nitrogen content of the Chlorella, 400 ml samples were taken from each replication. After being filtered they were carefully washed to remove any culture medium. The samples were then analyzed for total nitrogen by the micro-Kjeldahl method. The final results were obtained as milligrams of nitrogen per gram dry weight of Chlorella.

Lipid content. For lipid determinations, 800 ml samples of each replication were used. After being filtered, each sample was placed in a previously weighed, oven dried, extraction thimble. The dry weight of the alga was determined as described previously. The samples were then refluxed in a Soxhlet refluxer for 8 hours in a 1:3 ether-alcohol solvent. After the refluxing, the thimbles were dried and weighed. Lipid content was determined by subtracting the weight of thimble and alga after refluxing from total weight of the two before. The final results were expressed in grams of lipid per gram dry weight of Chlorella.

Radioisotope uptake by food chains. After two days the various trophic pools were separated into their components for counting.

Fish were removed, ground in a high speed blender and then washed onto filters. The zooplankton and alga were separated by a fine mesh net which allowed passage of algae but retained the zooplankton. After separation they were both washed onto filters. The filters were then mounted on planchets and oven dried. Planchets were counted in an internal proportional counter. No correction was made for self absorption. The final results were expressed as counts per minute per trophic level.

RESULTS

Radium uptake and concentration

Radium 226 was accumulated by all the algae tested; however, the amount of radium taken up was affected by the genus of the alga, the concentration of radium in the medium, and the length of time the alga was exposed to the radium. Analyses of variance indicated these three factors all had a highly significant effect on the amount of radium accumulated.

The initial experiment to determine radium uptake by Vaucheria used much higher concentrations of radium than were used in the later uptake experiments. This first experiment with Vaucheria used concentrations of 0, 5,000, 10,000, 20,000, and 40,000 pc Ra/l. In this experiment the analysis of variance indicated both length of exposure and concentration of radium were highly significant (Table 26).[4] Uptake of radium at these high concentrations had a linear relationship with the concentration of radium and a cubic relationship with time. The uptake of radium increased with each increase of radium in the medium and continued to do so up to 40,000 pc/l (Figure 2, Table 4). Radium uptake by the alga increased rapidly for the first 24 hours. In the next 24 hours the alga lost radium until it reached a concentration of radium which it approximately maintained until the experiment was terminated (Figure 3). This increase in radium concentration for 24 hours and then a decrease was the same in all concentrations of radium (Figure 4).

[4]All analysis of variance tables are in Appendix A.

Table 4. Mean values of radium uptake by Vaucheria in cpm per milligram ash weight as affected by concentration of radium 226 and length of exposure

Conc** (pc/l)	Time** (days)				
	½	1	2	4	Total
0	3.40	11.49	10.75	12.74	38.38
5,000	14.34	110.16	11.90	25.65	162.05
10,000	42.11	107.78	17.86	46.89	214.64
20,000	56.45	311.71	12.44	31.43	412.03
40,000	75.63	510.96	60.81	87.73	735.13
Total	191.93	1,052.10	113.76	204.44	

**Significant at 0.5 percent probability.

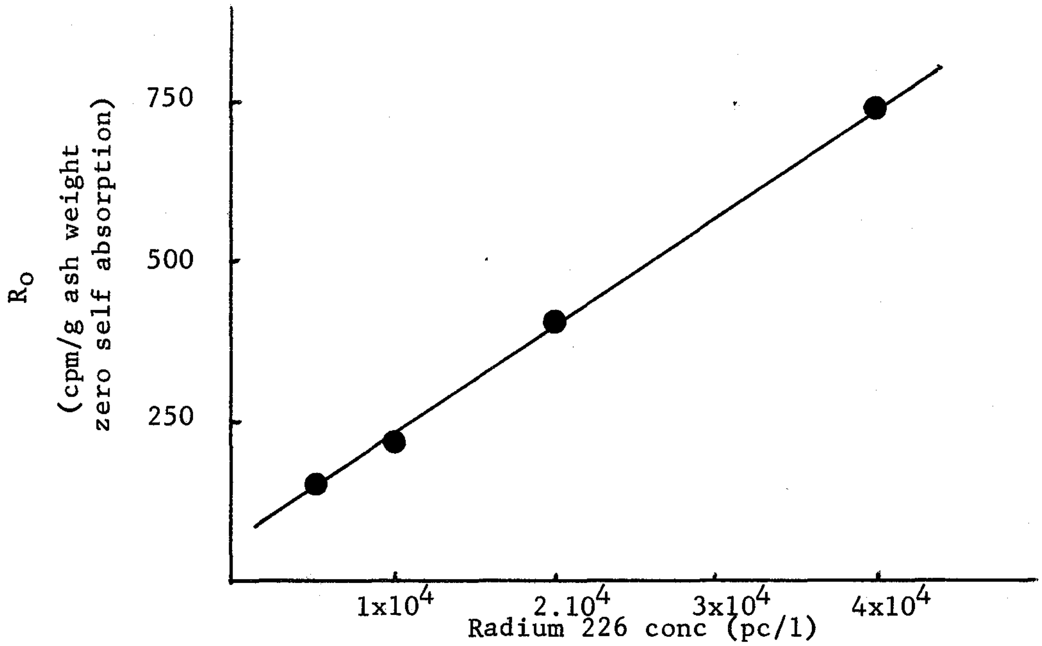

Figure 2. Relationship of radium 226 uptake by Vaucheria to radium concentration in the medium

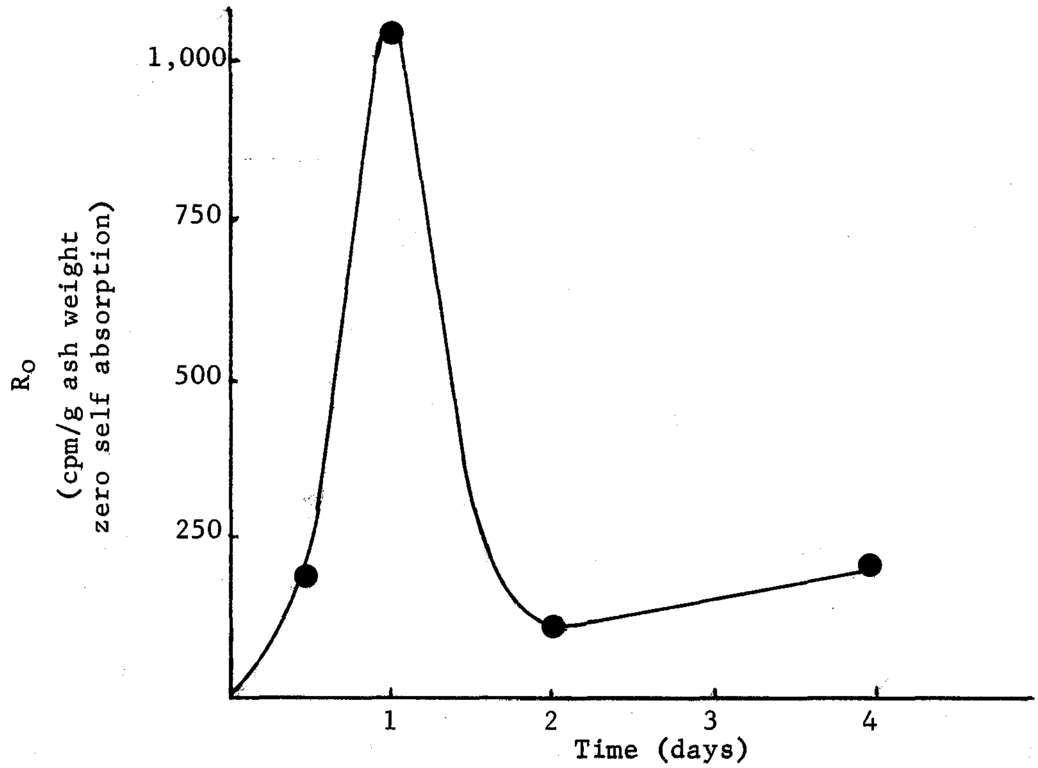

Figure 3. Relationship of radium 226 uptake by Vaucheria to length of exposure to radium

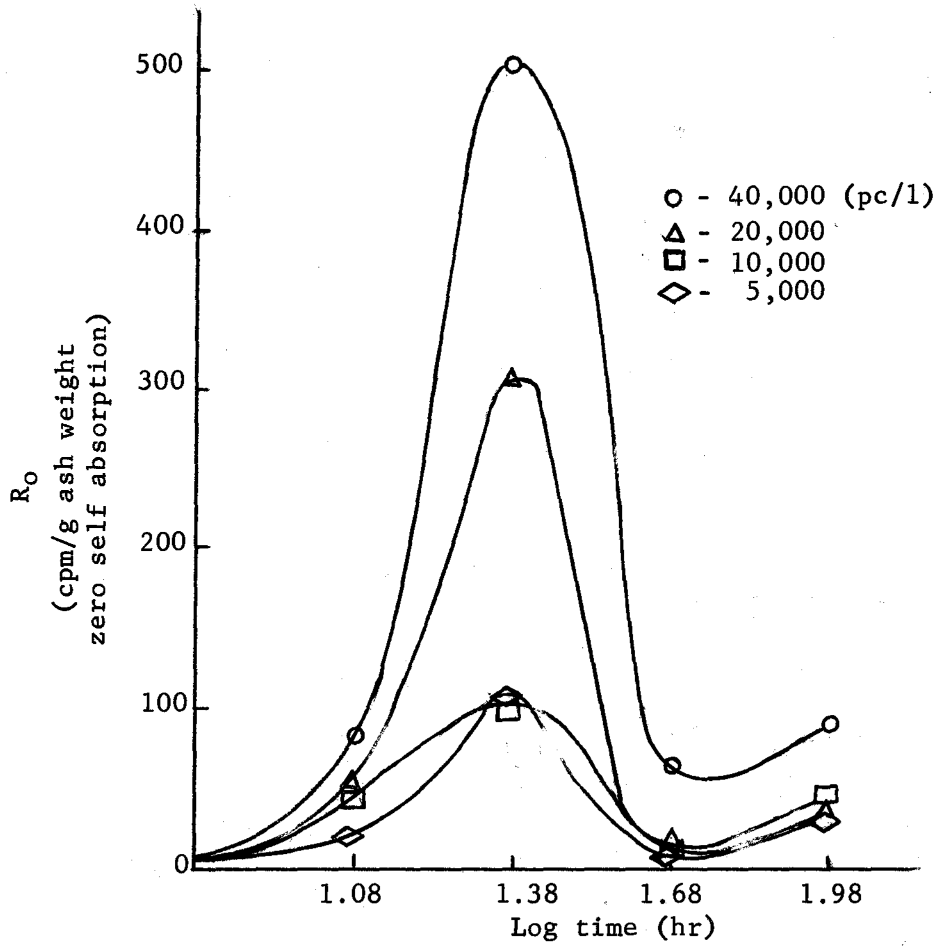

Figure 4. Relationship of radium 226 uptake by Vaucheria in four concentrations of radium at four time periods

The next uptake experiment with Vaucheria used radium concentrations of 0, 1, 5, 25, and 125 pc/l and was sampled just three times. The concentration of radium and the length of time the alga was exposed had highly significant effects on the uptake of radium (Table 27). Both factors had a linear relationship with the uptake of radium by Vaucheria. The more radium in the medium and the longer the alga was exposed, the more radium the alga accumulated (Table 5). Since three tanks were used as water baths for this experiment, the data were tested for significant differences due to tanks (Table 28). Results of the analysis of variance indicated there was no significant difference caused by the tanks so in all further analyses the tanks were grouped as replications.

The experiment to test differences in radium uptake due to type of alga demonstrated a highly significant effect due to algae (Table 29). The interactions between algae and radium concentration and algae and length of exposure also were shown to be very significant. The two algae, Vaucheria and Cladophora, appeared to take up the radium in somewhat similar manner on a dry weight basis (Figure 5). Both algae accumulated some radium from the lowest concentration with Vaucheria accumulating slightly more, but as the radium concentrations became greater than 25 pc/l, Cladophora accumulated more than Vaucheria (Table 6). Little radium was accumulated by either alga until the radium concentration reached 25 pc/l. It appeared the amount of radium accumulated was a function of the amount of radium present in the medium. Cladophora continued to accumulate radium throughout the experiment while Vaucheria began to lose radium after 24 hours (Figure 6). The radium content of Vaucheria was higher than Cladophora after 48 hours but by 96 hours Cladophora had accumulated more

Table 5. Mean values of radium uptake by Vaucheria in cpm per milligram ash weight as affected by concentration of radium 226 and length of exposure

Conc.** Ra 226 (pc/l)	Time** (days)			
	1	2	3	Total
0	2.973	0.500	0.459	3.932
1	1.319	0.671	8.211	10.201
5	1.056	0.958	16.828	18.842
25	1.603	2.921	3.006	7.530
125	6.734	11.196	24.293	42.223
Total	13.685	16.246	52.797	

**Significant at 0.5 percent probability.

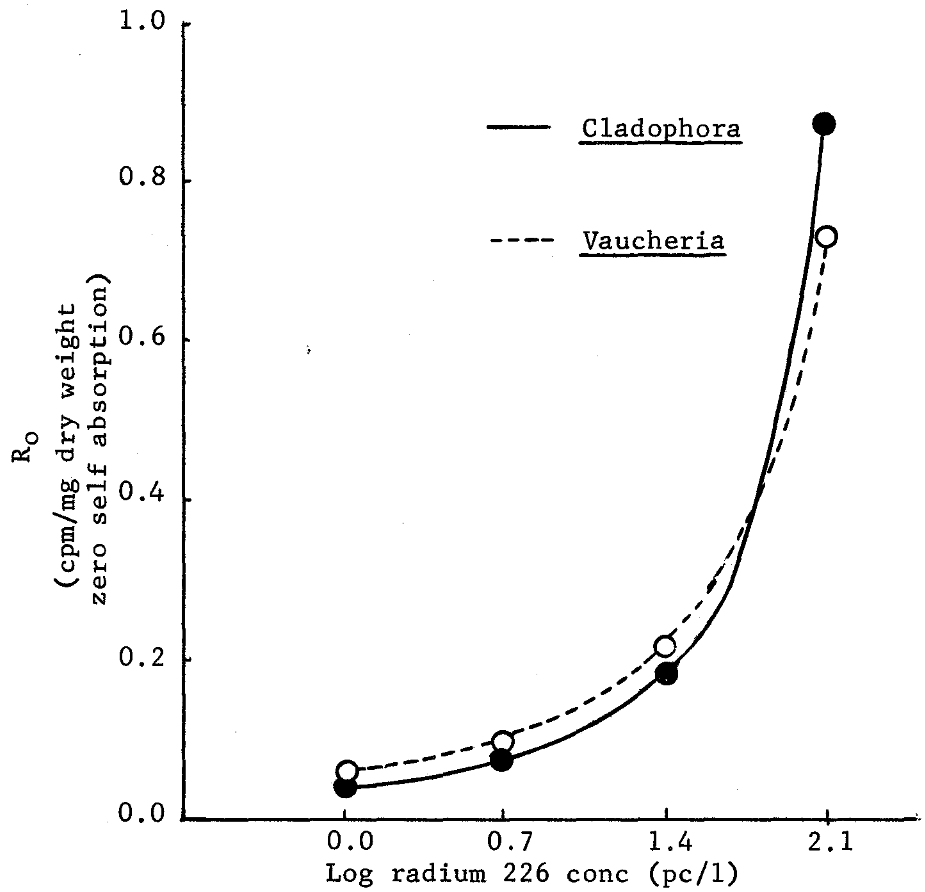

Figure 5. Comparison of radium 226 uptake by _Cladophora_ and _Vaucheria_ at four radium concentrations

Table 6. Table of mean values of radium uptake by Cladophora and Vaucheria in cpm per milligram ash weight as affected by concentration of radium 226 and length of exposure

Conc.** (pc/1)	Algae**										
	Cladophora					Vaucheria					Total
	Time (hrs.)**				Sub-total	Time (hrs.)**				Sub-total	
	12	24	48	96		12	24	48	96		
0	.0211	.0164	.0222	.0358	.0955	.0143	.0533	.0189	.0205	.1070	.2025
1	.0243	.0198	.0180	.0529	.1150	.0165	.0377	.0259	.0266	.1067	.2217
5	.0346	.0363	.0401	.0946	.2056	.0307	.0913	.0284	.0297	.1801	.3857
25	.0906	.1502	.1045	.2280	.5733	.0769	.0901	.0739	.0717	.3126	.8859
125	.4797	.7779	.5639	.9149	2.7364	.2010	.3889	.3829	.1651	1.1379	3.8743
Total	.6503	1.0006	.7487	1.3262		.3394	.6613	.5300	.3136		

**Significant at 0.5 percent probability.

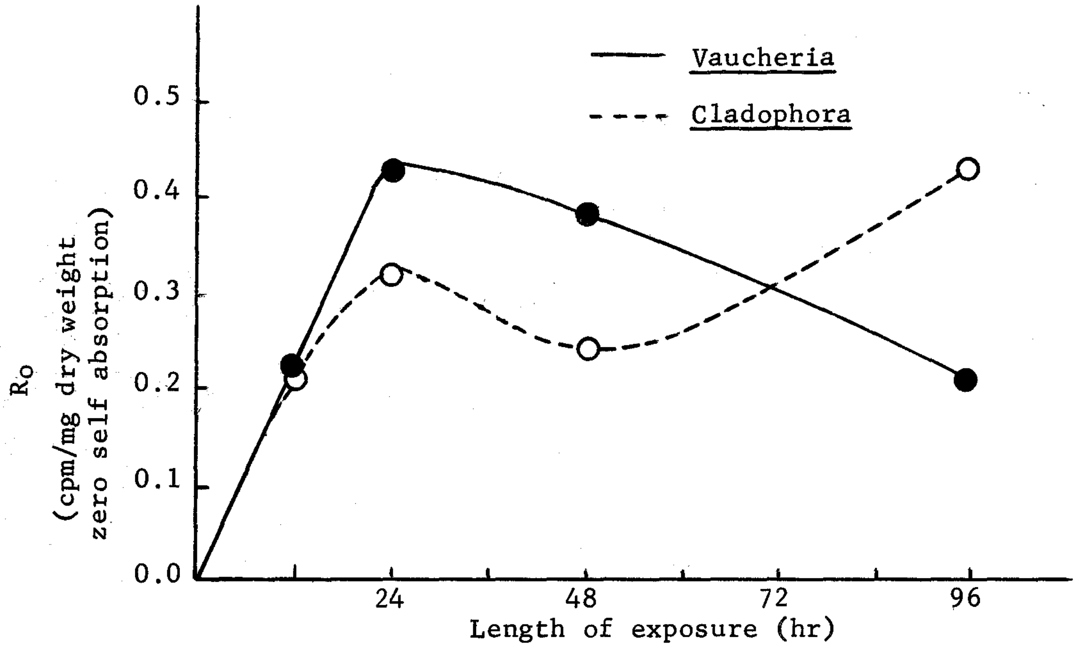

Figure 6. Comparison of radium 226 uptake by Cladophora and Vaucheria at different lengths of exposure

radium per gram dry weight than had Vaucheria.

Radium uptake by both Cladophora and Vaucheria from concentrations of radium up to 25 pc/l showed very little difference between alga, the radium concentration, or length of time the alga was exposed (Figure 7, 8). However, when the concentration of radium reached 125 pc/l there was a much greater radium accumulation by both algae. In Vaucheria the radium uptake increased for 24 hours in all concentrations of radium but after 24 hours the amount of radium accumulated by the algae decreased. Cladophora, in 125 pc/l, continued to accumulate radium for the duration of the experiment. It appeared from the data that possibly these algae required a certain amount of radium in their media before they could accumulate it to any degree. This "threshold" level seemed to be somewhere above 25 pc/l.

A third determination of radium uptake was made using the unicellular alga Chlorella. This alga was exposed to radium concentrations of 0, 6, 30, 150, and 750 pc/l. Radium uptake by Chlorella increased as the concentration of radium in the medium increased with the exception of 150 pc/l (Figure 9, Table 7). Though uptake from this radium concentration followed the same general pattern as the others, it never reached the levels expected. Again an analysis of variance showed both the length of exposure and the concentration of radium had a highly significant effect on radium uptake (Table 30). From Table 7 it was seen that the amount of radium per gram dry weight of alga increased up to the tenth or twentieth day and afterwards decreased. This was probably because the Chlorella cultures continued to divide throughout the experiment and as the numbers increased there was less radium available per unit of algae.

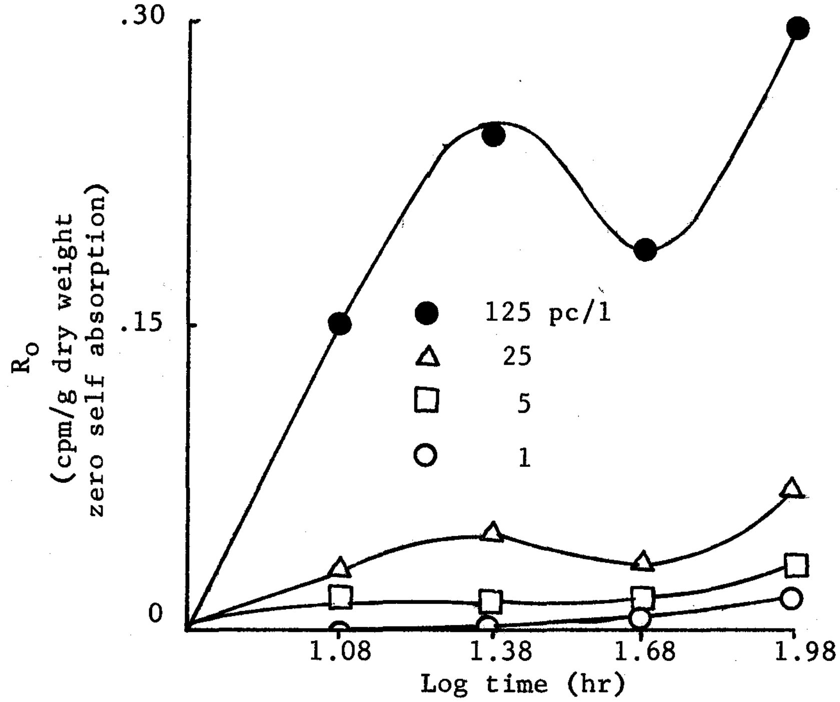

Figure 7. Comparison of radium 226 uptake by Cladophora in four different radium concentrations at four time periods

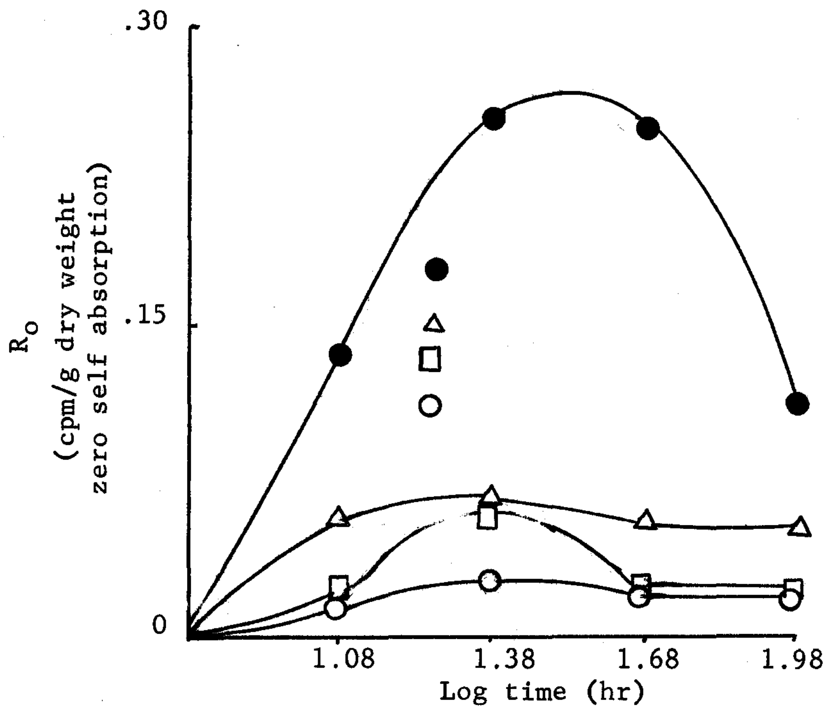

Figure 8. Comparison of radium 226 uptake by Vaucheria in four different radium concentrations at four time periods

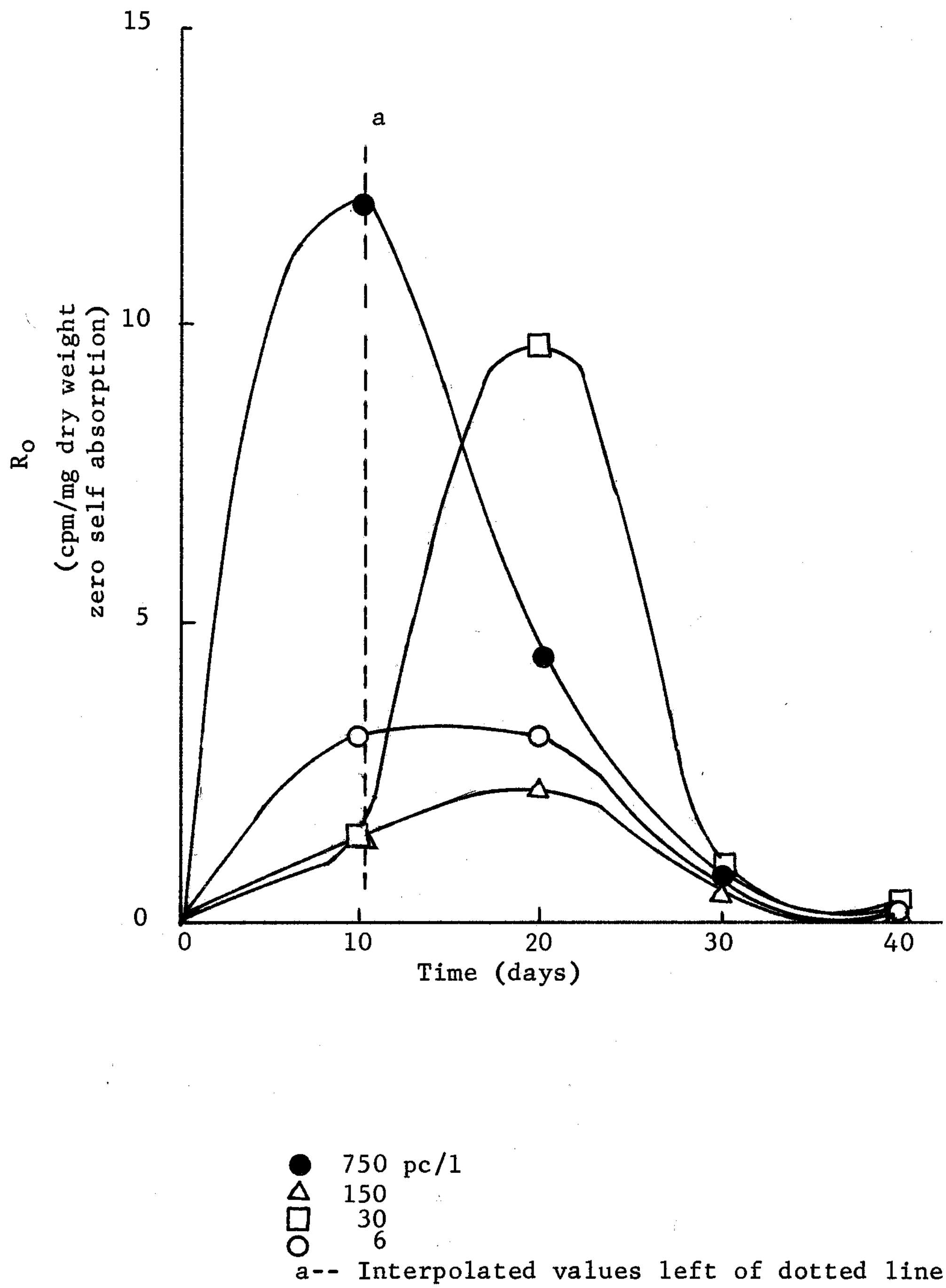

Figure 9. Comparison of radium 226 uptake by Chlorella in four different radium concentrations at four time periods

Table 7. Mean values of radium uptake by Chlorella in cpm per milligram dry weight as affected by concentration of radium 226 and length of exposure

Conc** Ra 226 (pc/1)	Time** (days)				Total
	10	20	30	40	
0	0.474	0.722	0.666	0.152	2.014
6	3.199	3.293	0.624	0.369	7.485
30	1.237	9.814	0.735	0.591	12.377
150	1.291	2.283	0.590	0.275	4.439
750	12.186	4.437	0.669	0.235	17.527
Total	18.387	20.549	3.284	1.622	

** Significant at 0.5 percent probability

In general, Chlorella appeared to follow the patterns of Vaucheria and Chadophora; however, this assumption may not be valid since the Chlorella was sampled at much longer time intervals than the other algae. The interaction surface of the radium uptake response demonstrated a general decrease of radium per gram algae through time and an increase with increased radium concentration (Figure 10). One exception occurred at 30 pc/l and 20 days where uptake was much higher with no apparent reason.

Concentration factors were determined for both the filamentous and unicellular algae in all uptake experiments (Tables 8, 9, 10). In the same concentration of radium Vaucheria tended to concentrate more radium than Cladophora for the first 24 hours but after 96 hours Cladophora had concentrated more radium (Figure 11). In the lower radium concentrations Cladophora concentrated radium as much as 248 pc/g dry weight and Vaucheria up to 226 pc/g dry weight. In the high radium concentrations Vaucheria concentrated radium as much as 19 x 10^4 pc/g dry weight. The highest radium concentration by Chlorella was 10 x 10^3 pc/g dry weight. The highest concentration factors were always found in the lowest radium concentrations and were higher for Chlorella in all instances than for the filamentous algae. Due to insufficient amounts of Chlorella, the count rate at zero self absorption was not determined for this alga, and therefore the calculations gave higher radium concentrations than actually occurred. It appeared from the data that Vaucheria had the ability to concentrate more radium from low radium concentrations than did Cladophora. The ability of Cladophora to concentrate radium did not vary much regardless of the concentration of radium in the medium.

In order to determine if there was a significant difference

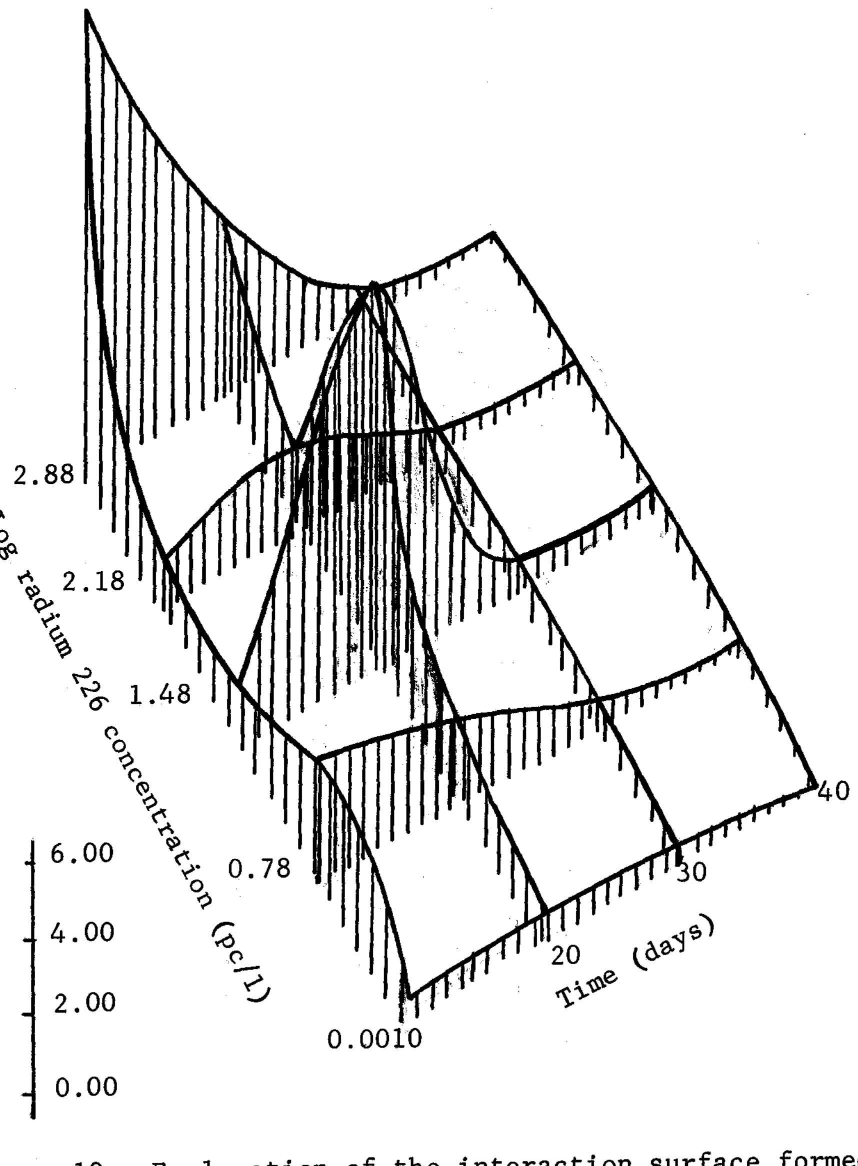

Figure 10. Exploration of the interaction surface formed by the radium 226 uptake response of <u>Chlorella</u> exposed to five treatment levels of radium for four time periods.

Table 8. Mean concentration factors for Vaucheria and Cladophora as affected by length of exposure and concentration of radium 226

		Cladophora		Vaucheria	
Length of exposure (hr)	Water (pc Ra/l)	Alga (pc Ra/g dry weight)	K[a]	Alga (pc Ra/g dry weight)	K[a]
12	0	0.00	0	0.00	0
	1	0.88	88	1.33	133
	5	3.79	76	9.53	191
	25	19.68	79	36.45	146
	125	129.87	104	108.61	87
24	0	0.00	0	0.00	0
	1	0.93	93	9.01	901
	5	5.61	112	31.16	623
	25	37.89	152	52.39	210
	125	215.62	173	226.16	181
48	0	0.00	0	0.00	0
	1	1.19	119	3.71	371
	5	6.26	125	5.64	113
	25	24.47	96	32.09	128
	125	154.59	124	211.75	169
96	0	0.00	0	0.00	0
	1	4.84	484	3.71	354
	5	16.62	332	5.05	101
	25	54.40	218	29.76	119
	125	248.92	199	84.07	67

[a] $K = \frac{\text{pc radium-226/g dry wt algae}}{\text{pc radium-226/g } H_2O}$ = concentration factor

Table 9. Mean concentration factors for *Vaucheria* as affected by length of exposure and concentration of radium 226 using the high concentrations of radium

Length of exposure (hr)	Water (pc Ra/1)	Alga (pc Ra/g dry weight)	K[a]
	0	0	0
	5,000	4,175	835
12	10,000	5,234	523
	20,000	11,720	586
	40,000	27,548	688
	0	0	0
	5,000	37,625	7,525
24	10,000	36,716	3,671
	20,000	114,463	5,723
	40,000	190,426	4,760
	0	0	0
	5,000	431	86
48	10,000	2,716	271
	20,000	643	32
	40,000	19,083	477
	0	0	0
	5,000	4,917	983
96	10,000	13,004	1,300
	20,000	7,115	355
	40,000	28,582	714

[a]$K = \dfrac{\text{pc radium-226/g dry wt algae}}{\text{pc radium-226/g } H_2O}$ = concentration factor

Table 10. Concentration factors for <u>Chlorella</u> as affected by length of exposure and concentration of radium 226

Length of exposure (days)	Water (pc Ra/l)	Alga (pc Ra/g dry weight)	K[a]
10	0	0	0
	6	2,412	402,000
	30	675	22,449
	150	723	4,820
	750	10,365	13,816
20	0	0	0
	6	2,275	379,167
	30	8,046	268,197
	150	1,382	9,213
	750	3,288	4,382
30	0	0	0
	6	228	38,000
	30	327	10,899
	150	198	7,320
	750	268	357
40	0	0	0
	6	192	32,000
	30	388	12,933
	150	109	726
	750	74	98

[a]$K = \frac{\text{pc radium-226/g dry wt algae}}{\text{pc radium-226/g } H_2O}$ = concentration factor

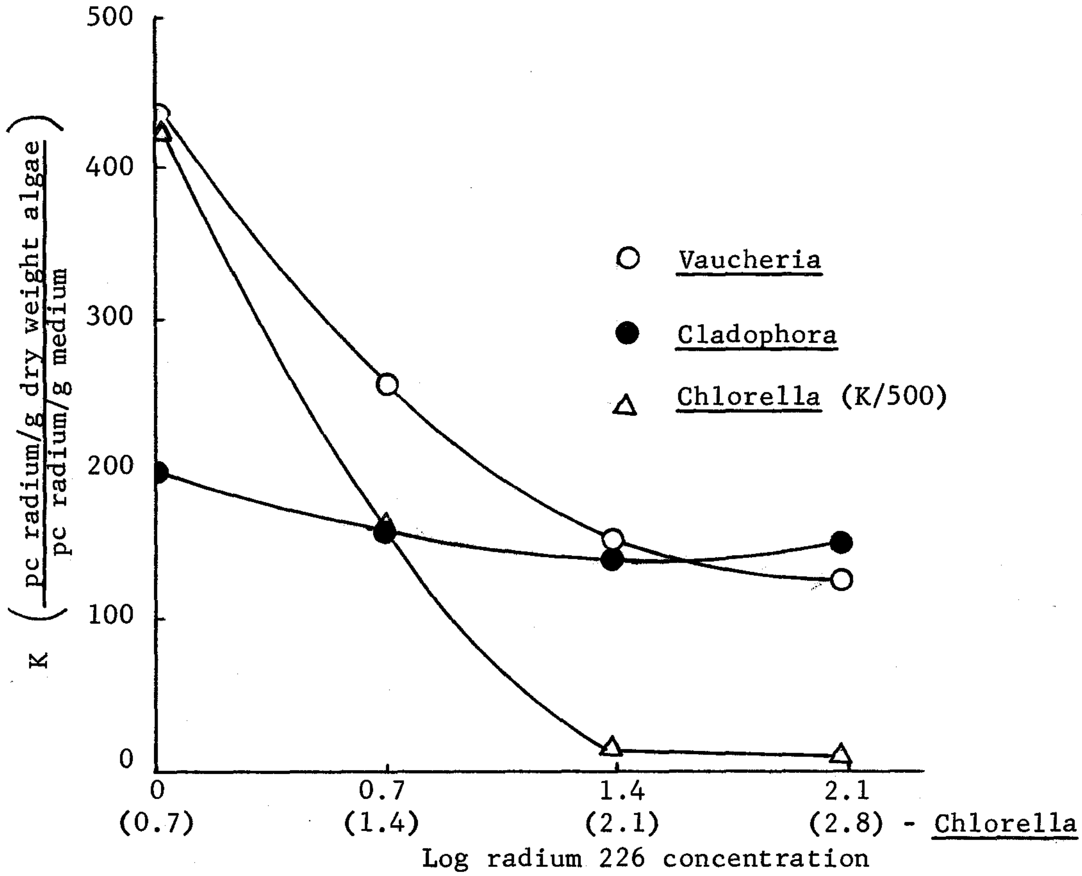

Figure 11. Relationship of concentration factors of *Cladophora*, *Vaucheria*, and *Chlorella* to the concentration of radium 226 in the medium

between the abilities of Cladophora and Vaucheria to concentrate radium, a t-test was run on their mean concentration factors (Table 45). The test showed no significant difference between the concentration factors of the two algae.

The data suggested that radium uptake by Vaucheria may have a completely linear relationship with the concentration of radium in the medium and length of exposure so analyses of covariance were used to test this hypothesis (Table 47). The data were tested for a common regression slope to determine if they could all fit the same line. The analyses indicated there was no difference in radium uptake by Vaucheria between the four low concentrations of radium: 1, 5, 25, and 125 pc/1, over the four time periods or between the four high concentrations: 5,000, 10,000, 20,000, and 40,000 pc/1, over the four time periods. At 12 hours and 48 hours the radium uptake by Vaucheria had the same slope over all eight concentrations; however, there was a significant difference between slopes at 12 and 96 hours.

Effect of radium on oxygen production by algae

Gross oxygen production, net oxygen production, and respiration by Vaucheria and Cladophora were measured and analyzed to determine if they were affected by radium 226. These parameters were measured on algae exposed to radium concentrations of 0, 1, 5, 25, and 125 pc/1 at 12, 24, 48, and 96 hours.

In Vaucheria the effect of length of exposure on gross oxygen production was highly significant, but the concentration of radium was only significant at the 10 percent level of probability (Table 31). The relationship of length of exposure to gross oxygen production was linear while the relationship of radium concentration to gross oxygen production was quadratic. The mean gross oxygen production values decreased as the concentration of radium increased except for the highest radium concentration where the gross production increased (Table 11, Figure 12).

Analysis of variance of the effects of radium concentration and length of exposure on respiration of Vaucheria again demonstrated the length of exposure had a more significant effect than the concentration of radium; however, they both were significant (Table 32). Length of exposure and concentration of radium each had a quadratic relationship with respiration. Mean values showed Vaucheria respired less in the intermediate concentrations of radium than it did in the control or highest radium concentration (Table 12, Figure 12).

Net oxygen production of Vaucheria was often negative (Table 13). The analysis of variance demonstrated that length of exposure had a highly significant effect on net oxygen production but the effect of the radium concentration was not significant (Table 33). Since net oxygen production is the difference between gross production and respiration, when respiration became greater than gross production, net production became negative (Figure 12). When Vaucheria was exposed to radium concentrations of 5 pc/1 or more, either gross oxygen production was retarded or respiration was increased, giving a negative net oxygen production.

In Cladophora the length of exposure had a highly significant effect upon gross oxygen production but no effect was attributed to the concentration of radium (Table 34). There was very little change in mean values of gross oxygen production over the different radium concentrations,

Table 11. Mean values of gross production by Vaucheria in ppm oxygen per gram dry weight as affected by concentration of exposure

Conc* Ra 226 (pc/1)	Time** (days)				Total
	1/2	1	2	4	
0	.1057	.0883	.2952	.0589	.5481
1	.0676	.0221	.1587	.0373	.2857
5	-.0119	.2628	.0757	-.0756	.2510
25	.0443	.1124	.0004	-.0369	.1202
125	.3137	.2433	-.0558	-.0124	.4888
Total	.5194	.7289	.4742	-.0287	

** Significant at 0.5 percent probability
* Significant at 10.0 percent probability

Table 12. Mean values of respiration by Vaucheria in ppm oxygen per gram dry weight as affected by concentration of radiom 226 and length of exposure

Conc* Ra 226 (pc/1)	Time** (days)				Total
	1/2	1	2	4	
0	.0077	.1058	.2739	.0415	.4289
1	-.0906	.0891	.1087	.0531	.1603
5	-.0791	.2872	.1551	-.0089	.2643
25	.0348	.1292	.0509	-.0175	.1974
125	.2033	.2553	.1332	-.0033	.5885
Total	.0761	.8666	.7218	-.0251	

** Significant at 0.5 percent probability
* Significant at 2.5 percent probability

Table 13. Mean values of net production by *Vaucheria* in ppm oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Conc Ra 226 (pc/l)	Time** (days)				Total
	1/2	1	2	4	
0	.0887	.0087	-.0044	.0174	.1192
1	.1582	.0359	.0446	-.1133	.1254
5	.0654	-.0244	-.0731	.0188	-.0133
25	.0095	-.0189	-.0422	-.0256	-.0772
125	.1104	-.0120	-.1379	-.0602	-.0997
Total	.4322	-.0107	-.2130	-.1629	

although it decreased slightly at first and then increased in the higher radium concentrations (Table 14, Figure 13).

Respiration of Cladophora, however, was greatly affected by the concentration of radium as well as by the length of exposure (Table 35). Mean values for respiration in the different radium concentrations showed little difference except for the increase at the highest radium concentration (Table 15, Figure 13).

Analysis of the net oxygen production of Cladophora found both the concentration of radium and the length of exposure had significant effects (Table 36). As in the case of Vaucheria there again appeared negative net production (Table 16, Figure 13).

A comparison of Figures 12 and 13 seemed to indicate that Cladophora was somewhat more resistant to the effects of radium than was Vaucheria. Cladophora continued a positive net oxygen production over a much wider range of radium concentrations than did Vaucheria. Both algae seemed to be affected more by the length of time they were exposed than by the concentration of radium. The net oxygen production of both algae plotted against time showed net oxygen production became negative between 12 and 24 hours (Figures 14, 15). Part of this significant effect might have been indicative of an effect due to transplanting the algae from their natural habitat; however, since the mean net production values for the controls were positive, the greatest effect was due to the radium.

Analyses of variance indicated there was a significant difference between replications for all the measurements except gross oxygen production in Vaucheria. Since the gross oxygen figures were calculated from respiration and net oxygen measurements it was thought, in this

Table 14. Mean values of gross production by Cladophora in ppm oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Conc Ra 226 (pc/l)	Time** (days)				Total
	1/2	1	2	4	
0	1.0554	.1829	.2459	.0554	1.5396
1	1.1327	.1599	.2032	.1403	1.3557
5	.8396	-.0839	.3096	.0660	1.1313
25	1.2452	-.0203	.0686	.0803	1.3738
125	1.1170	-.0289	.1822	.0817	1.3520
Total	5.3899	.2097	1.0095	.4237	

** Significant at 0.5 percent probability

Table 15. Mean values of the respiration by *Cladophora* in ppm oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Conc** Ra 226 (pc/1)	Time** (days) 1/2	1	2	4	Total
0	.5240	.3385	.2112	.0771	1.1508
1	.5815	.1350	.1779	.2322	1.1266
5	.3965	.1416	.2847	.2591	1.0819
25	.4861	.1811	.1237	.3140	1.1049
125	.6054	.3484	.1877	.3814	1.5229
Total	2.5935	1.1446	.9862	1.2638	

** Significant at 0.5 percent probability

Table 16. Mean values of the net production by <u>Cladophora</u> in ppm oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Conc* Ra 226 (pc/l)	Time** (days)				Total
	1/2	1	2	4	
0	.5315	-.1556	.0347	-.0217	.3889
1	.5513	.0249	.0254	-.0919	.1611
5	.4431	-.2254	.0250	-.1930	.0497
25	.7591	-.2014	-.0551	-.2336	.0269
125	.5116	-.3773	-.0107	-.2998	-.1762
Total	2.7966	-.9846	.0193	-.8400	

** Significant at 0.5 percent probability
* Significant at 5.0 percent probability

case, the variation in net production and respiration cancelled each other out. This difference in replications might have been because the algae used were of a different age or in a different physical condition.

The error term in these same analyses contained a large portion of the variance of the experiment. This indicated that either some of the factors affecting oxygen production in algae were not measured (there was a large variation between experimental units), or there was an error in technique. As a check on technique, duplicate samples were taken for dissolved oxygen determinations at each sampling period. Though there was no significant difference between determinations in the different analyses, a coefficient of variation was made for each experiment (Table 17). This demonstrated the wide variation between determinations and also showed how net production measurement had much more variation than gross production or respiration measurements. The greater variation in net production was because it was calculated from the respiration and gross production measurements and at times their variation was additive.

It was postulated that two things caused the variation between the dissolved oxygen sample readings. First, because the macrocosms used in the experiments were air-tight, the evolution of gases created a pressure in the system. When the water sample was removed it was super-saturated with gas and a combination of loss of pressure and turbulence while entering the bottle caused different amounts of gas to be lost from each sample. Secondly, once a water sample had been taken, a vacuum or negative pressure was created in the system. This in turn caused changes in the dissolved oxygen content of the water for the

Table 17. Coefficients of variation for three measurements on Cladophora and Vaucheria that were used to determine effects of radium 226 on productivity

Measurement	Coefficient of variation (%)	
	Vaucheria	Cladophora
Respiration	103	29
Gross oxygen production	124	45
Net oxygen production	861	401

next sample. However, since the difference between determinations was not significant, this variation had no effect on the final analyses.

The concentration of radium and the length of time the alga was exposed to the radium both had significant effects on the net oxygen production of Chlorella (Table 37). With this unicellular alga the concentration of radium had a more significant effect than it did with the filamentous algae. Mean values of net oxygen production in the different radium concentrations showed a decrease of oxygen production between the control and all radium concentrations (Figure 16, Table 18). The interaction surface formed by net oxygen production response for the five radium concentrations and four lengths of exposure demonstrated a general decrease in oxygen production after 10 days and in radium concentrations over 6 pc/l. However, it appeared that the oxygen response became similar in all treatment levels after 20 days.

Effect of radium 226 on carbon 14 uptake by algae

Type of algae, concentration of radium, and length of exposure to radium all had highly significant effects on the productivity of algae when it was measured as carbon 14 uptake (Table 38).

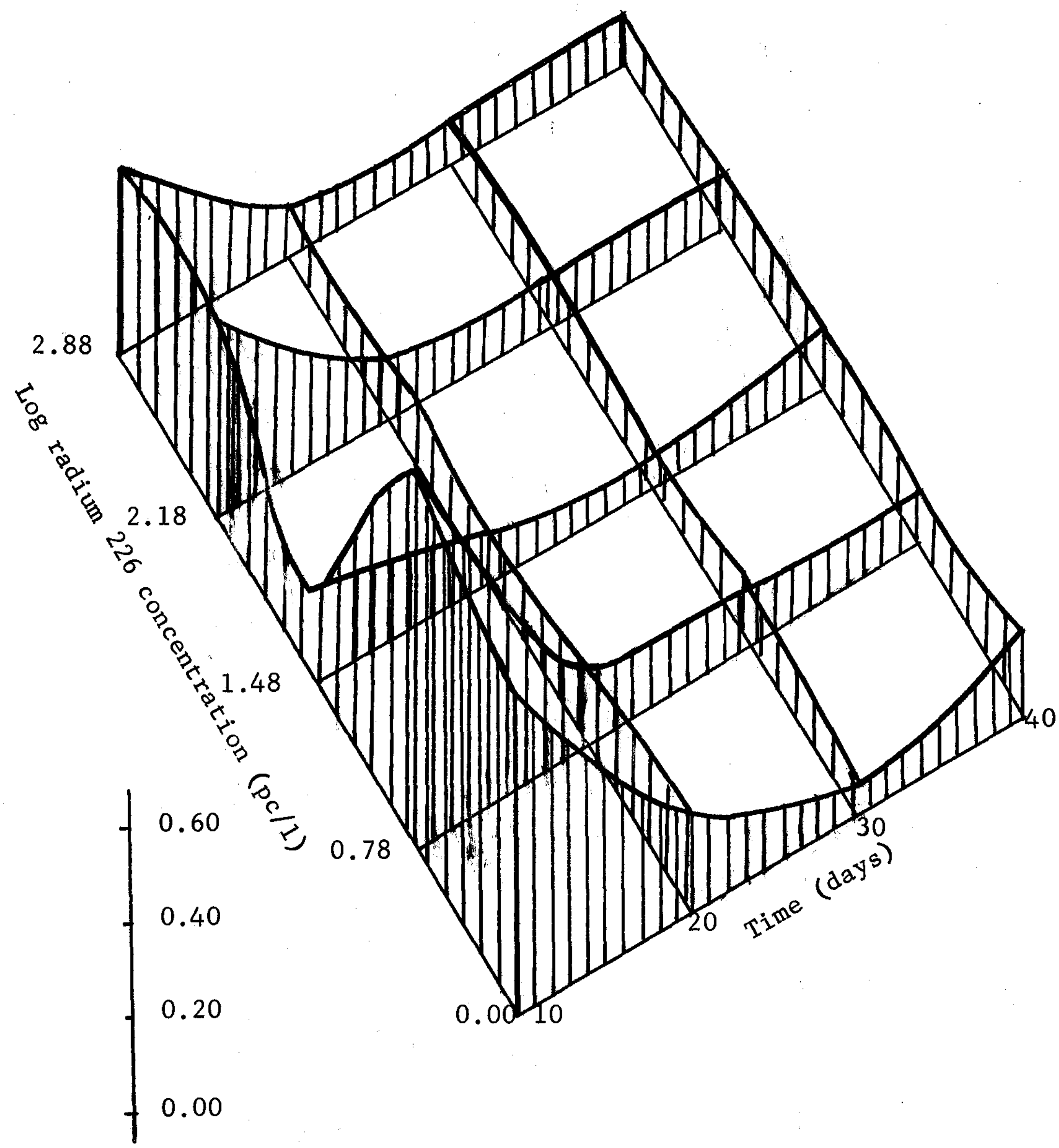

Figure 16. Exploration of the interaction surface formed by the oxygen production response of <u>Chlorella</u> exposed to five treatment levels of radium for four time periods

Table 18. Mean values of net production by <u>Chlorella</u> in ppm oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Conc** Ra 226 (pc/1)	Time* (days) 10	20	30	40	Total
0	.6861	.1935	.0550	.0902	1.8368
6	.7936	.1634	.1223	.1037	1.1830
30	.1671	.0911	.0583	.1344	0.4509
150	.3975	.1134	.1159	.0960	0.7228
750	.3874	.0942	.0872	.1109	0.6796
Total	2.4317	0.6556	0.4387	0.5352	

** Significant at 1.0 percent probability.
* Significant at 5.0 percent probability.

From the mean carbon 14 uptake values, it appeared that both Cladophora and Vaucheria had a similar type of response to radium, but the degree of response differed (Table 19). To test this, the significance of the regression components was established and it was found that in both algae the concentration of radium had a quadratic relationship and length of exposure had a linear relationship with carbon 14 uptake (Tables 39, 40). Regression lines fit to the data showed the carbon uptake by the two algae had almost identical responses to the same radium concentration (Figures 17, 18). The main difference observed was that Cladophora consistently had a higher uptake of carbon than did Vaucheria. Reference to the data in oxygen production (Figures 12, 13) demonstrated these results were similar to the results from the oxygen measurements. Cladophora maintained a positive net oxygen production over a wider range of radium concentrations and demonstrated a greater gross production than did Vaucheria. Since carbon 14 uptake measurements estimate production somewhere between gross and net oxygen production, it followed that Cladophora should have the higher carbon uptake. Vaucheria, however, was more resistant to the effects of radium over a period of time (Figure 18). Carbon uptake by Cladophora decreased the longer it was exposed to radium while Vaucheria maintained a steady increase in carbon uptake throughout the experiment. When the two algae were examined at each concentration of radium, again Vaucheria tended to increase in carbon 14 content throughout, while Cladophora decreased in carbon 14 content (Figures 19, 20). It appeared, however, from the controls, that this effect of time had little to do with the length of exposure to radium. It was more likely that the decrease in carbon

Table 19. Mean values of carbon 14 uptake by Cladophora and Vaucheria as cpm per gram dry weight as affected by concentration of radium 226 and length of exposure

Conc** Ra 226 (pc/l)	Algae**										Total
	Vaucheria					Cladophora					
	Time (hr)**					Time (hr)**					
	12	24	48	96	Sub-total	12	24	48	96	Sub-total	
0	124.1	287.5	352.2	245.4	1009.1	600.9	546.5	324.8	146.9	1619.1	2628.2
50	180.0	145.6	259.7	381.9	967.3	453.8	325.2	329.1	191.1	1299.1	2266.4
150	89.8	131.5	110.8	105.9	437.8	358.1	367.3	208.4	146.9	1080.5	1518.4
450	187.4	129.1	259.9	220.4	796.7	649.3	432.9	470.3	154.1	1706.6	2503.3
1350	246.1	261.3	172.8	330.8	1011.1	753.8	345.6	295.5	139.8	1534.7	2545.7
Total	827.3	954.8	1155.5	1284.4		2815.8	2017.4	1627.9	778.8		

**Significant at 0.5 percent probability.

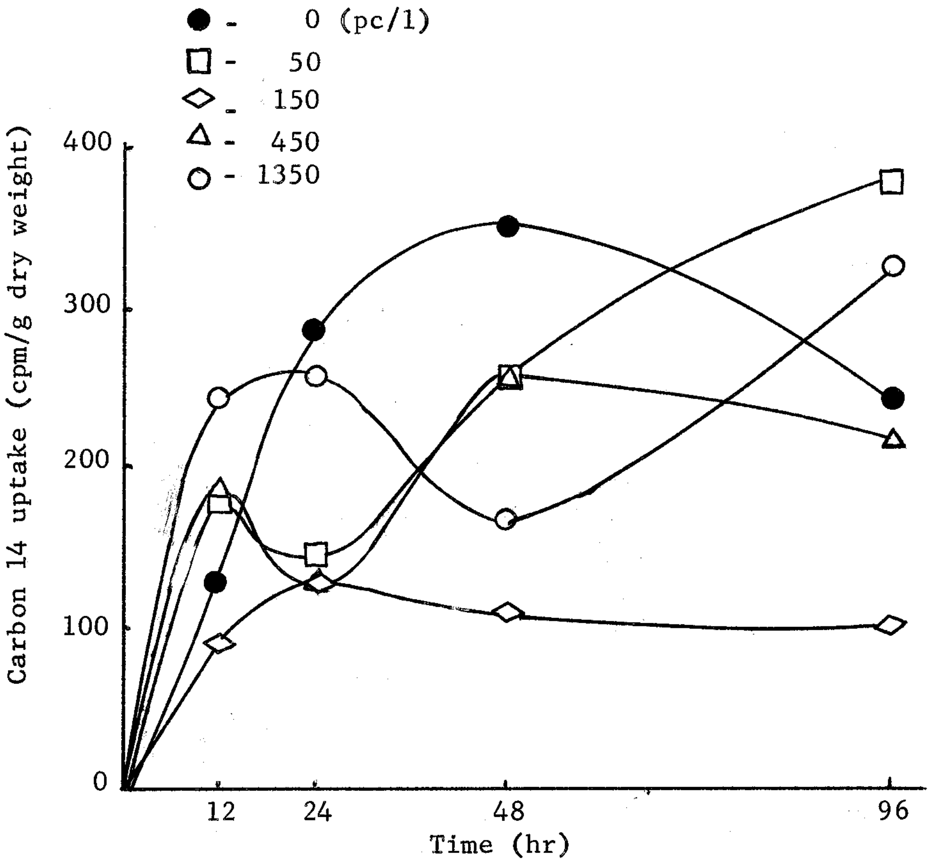

Figure 19. Relationship of carbon 14 uptake by Vaucheria to length of exposure in different concentrations of radium 226

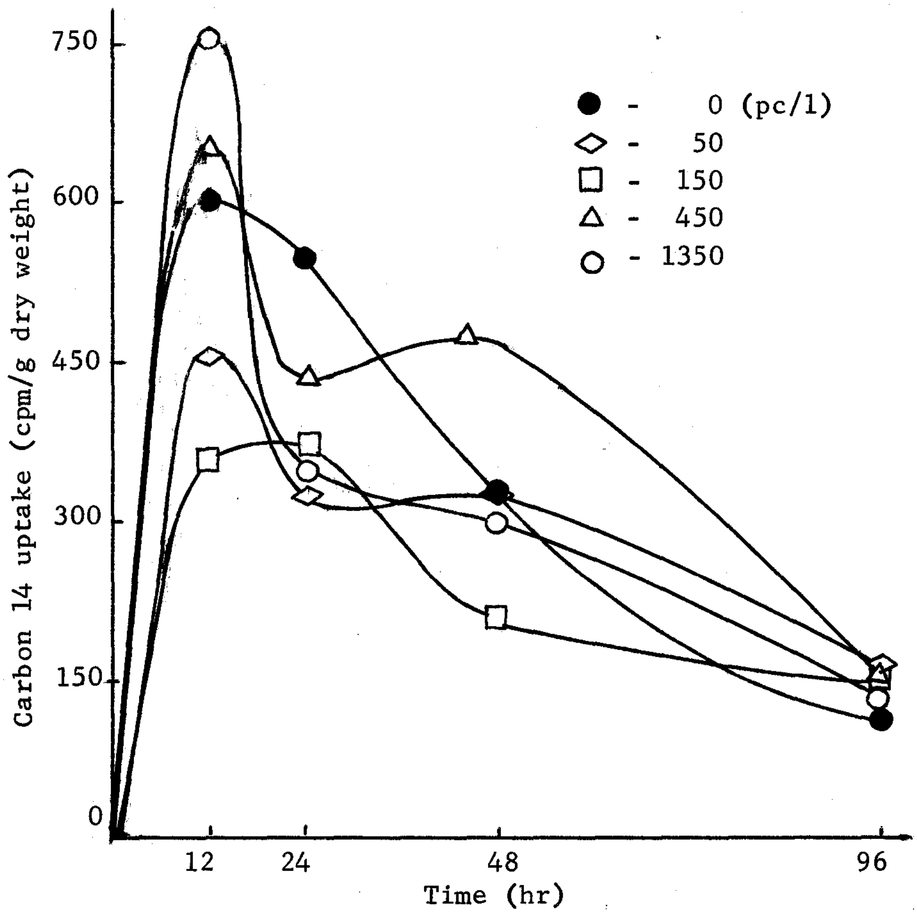

Figure 20. Relationship of carbon 14 uptake by Cladophora to length of exposure in different concentrations of radium 226

fixation was due to the artificial conditions under which the algae were placed. This was evident from the fact the controls reacted the same as the algae exposed to radium, except for the degree of response.

Carbon 14 uptake in _Chlorella_ was significantly affected by the length of exposure but the concentration of radium had no significant effect on it (Table 41). Here again the control and all concentrations of radium displayed a similar pattern. There was a decrease of carbon uptake for the first 30 days after which carbon uptake began to increase (Table 20, Figure 21). When carbon 14 uptake was compared with net oxygen production, both demonstrated a similar pattern (Figure 22).

Table 20. Mean values of carbon 14 uptake by _Chlorella_ in cpm per gram dry weight as affected by concentration of radium 226 and length of exposure

Conc. RA 226 (pc/1)	Time ** (days)				
	10	20	30	40	Total
0	139.28	41.93	25.62	95.85	302.68
6	123.75	40.31	30.36	53.69	248.11
30	118.30	28.48	30.76	77.50	256.04
150	104.32	67.01	25.65	99.71	296.69
750	144.88	69.02	20.22	49.52	283.64
Total	630.53	247.75	132.61	376.27	

**Significant at 0.5 percent probability.

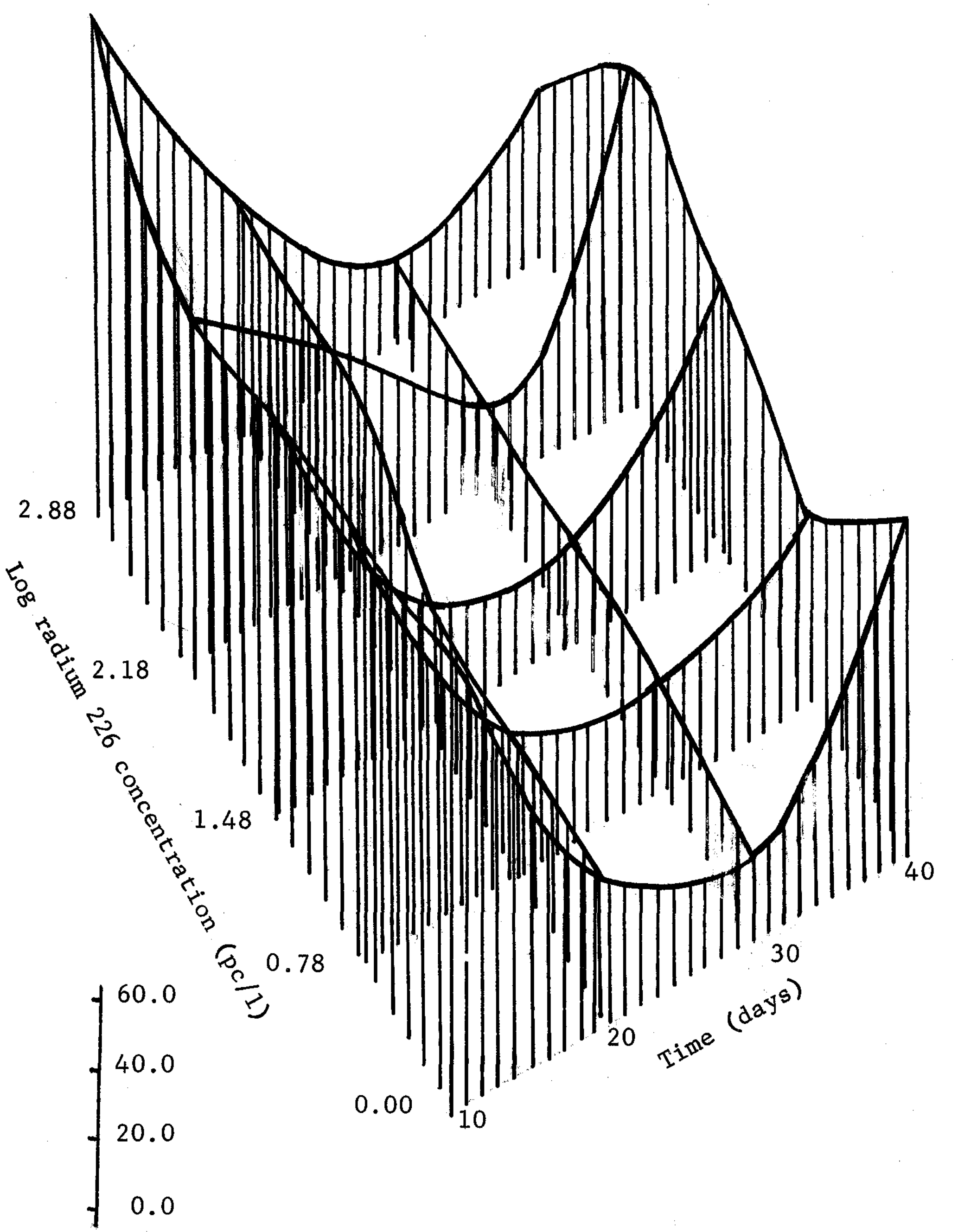

Figure 21. Exploration of the interaction surface formed by the carbon 14 uptake response of Chlorella exposed to five treatment levels of radium for four time periods.

Effects of radium 226 on unicellular algae

The concentration of radium in the medium had a significant effect on total nitrogen content, net oxygen production, and radium uptake of *Chlorella* (Tables 30, 37, 47). The length of exposure had a significant effect on the same parameters and also on the dry weight, the lipid content, and carbon 14 uptake of *Chlorella* (Tables 41, 43, 44).

Correlation coefficients indicated a statistically significant linear relationship between several of the measured parameters (Table 21). Oxygen production and carbon 14 uptake were statistically dependent upon each other and upon the total nitrogen content, the lipid content, and the dry weight of *Chlorella*. Radium uptake was significantly related to just the dry weight of the alga.

Passage of radium through a food chain

A comparison was made of the percent of carbon 14 and radium 226 passed through a simple food chain based on the assumption that carbon 14 would give a true picture of passage from primary producers to consumers. No statistical analysis was made of the data but the data clearly demonstrated the fact that a greater percentage of radium from labeled algae reached fish than from carbon 14 labeled algae (Figure 23). The percent of radium remaining appeared to have a linear relationship with the level of the food chain while carbon 14 had a nearly geometric relationship.

Table 21. Correlation coefficients among six parameters measured on <u>Chlorella</u> exposed to five concentrations of radium 226 for four lengths of time

	X_2	X_3	X_4	X_5	X_6
X_1	-.8486**	.5308*	.4902*	-.1557	-.3110
X_2		-.6170**	-.5161*	.1345	.1818
X_3			.5641**	.2353	-.5426*
X_4				.1644	-.7500**
X_5					-.4727*

** Significant at 1.0 percent probability
 * Significant at 5.0 percent probability

X_1 - Total nitrogen (mg/g dry weight algae)

X_2 - Lipids (g/g dry weight algae)

X_3 - C_{14} uptake (cpm/mg dry weight algae)

X_4 - Oxygen production (ppm/mg dry weight algae)

X_5 - Radium uptake (cpm/mg dry weight algae)

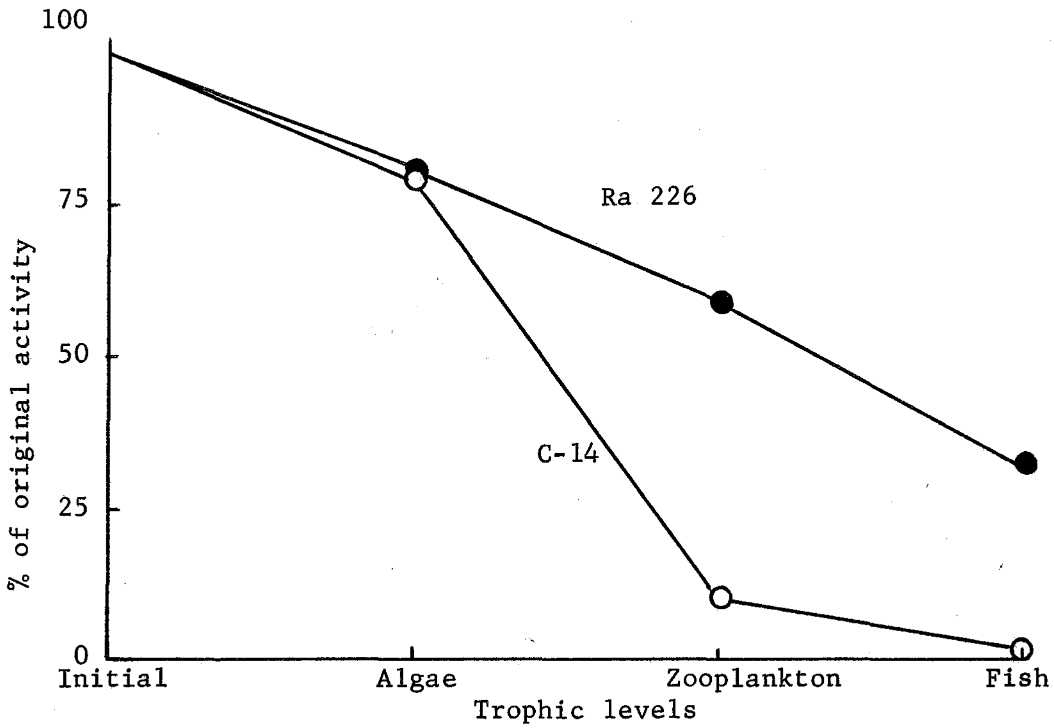

Figure 23. Comparison of the percent of carbon 14 and radium 226 from labeled algae remaining in the trophic levels of a simple food chain

DISCUSSION

Radium uptake and concentration

Results of the experiments clearly indicated that radium was accumulated by the algae tested. Uptake of radium by algae had been reported previously; however, except for values from the rivers in question, the concentration values here indicated algae are capable of accumulating more radium than had previously been suspected.

The mechanisms of the uptake of radionuclides are not clear, but in general may be explained by one or more of three major processes: adsorption to exposed surfaces, absorption into tissues, or through metabolic uptake.

It appeared likely that sorption played the most important role in the accumulation of radium since there is no known metabolic requirement for it. However, metabolic processes may have been a factor since it was reported that radium sometimes substitutes for calcium in metabolism (Eisenbud, 1963). Calcium, an essential element, is usually found as calcium pectate in cell walls or else as a phospholipid in protoplasmic membranes or adsorbed to proteins. Eppley (1938a) reported that calcium was bound to extracellular material in which case it would be difficult to distinguish from that which is adsorbed. An attempt was made to identify sites of radium attachment by separating the components of the cells. Radium was found in all fractions but, because radium adheres so strongly to surfaces, it was felt contamination of various components probably occurred during fractionation of the cells. If metabolism had been the most important pathway for radium accumulation, the radium should have been accumulated in the algae in approximately the same proportions as the radium-calcium ratio of the water. Uptake of elements in both plants and animals has been shown to depend on their ratio with elements of similar properties. Rice (1956) observed that strontium uptake by Carteria depended upon the strontium-calcium ratio of the water and Prosser (1945) reported the same thing for goldfish. On this basis the amounts of radium accumulated by the algae would have been much less than they were since calcium in the water was about 45 ppm while radium never was more than 125×10^{-9} ppm.

A logarithmic plot of algal radium content against concentration of radium resembled the Freundlich adsorption isotherm so the regression equations were calculated (Figure 24). If the slope of the lines had been unity it would have indicated the accumulation was not due to adsorption; however, since the slopes were less than one, radium accumulation could have been due to adsorption. Although the data were fit to the adsorption isotherm, they more closely fit a straight line relationship (Figure 2, 25). The relationship of uptake and external concentration may depart from the hyperbolic relationship expected when the uptake depends on the synthesis of new binding sites because adsorption sites are saturated, where diffusion limits ion transport, or when part of the uptake is passive mass flow. When uptake becomes proportional to concentration, as it did here, diffusion and mass flow are usually involved. However, in this instance, due to the very low radium concentrations used, there were probably an excess of binding sites available for adsorption.

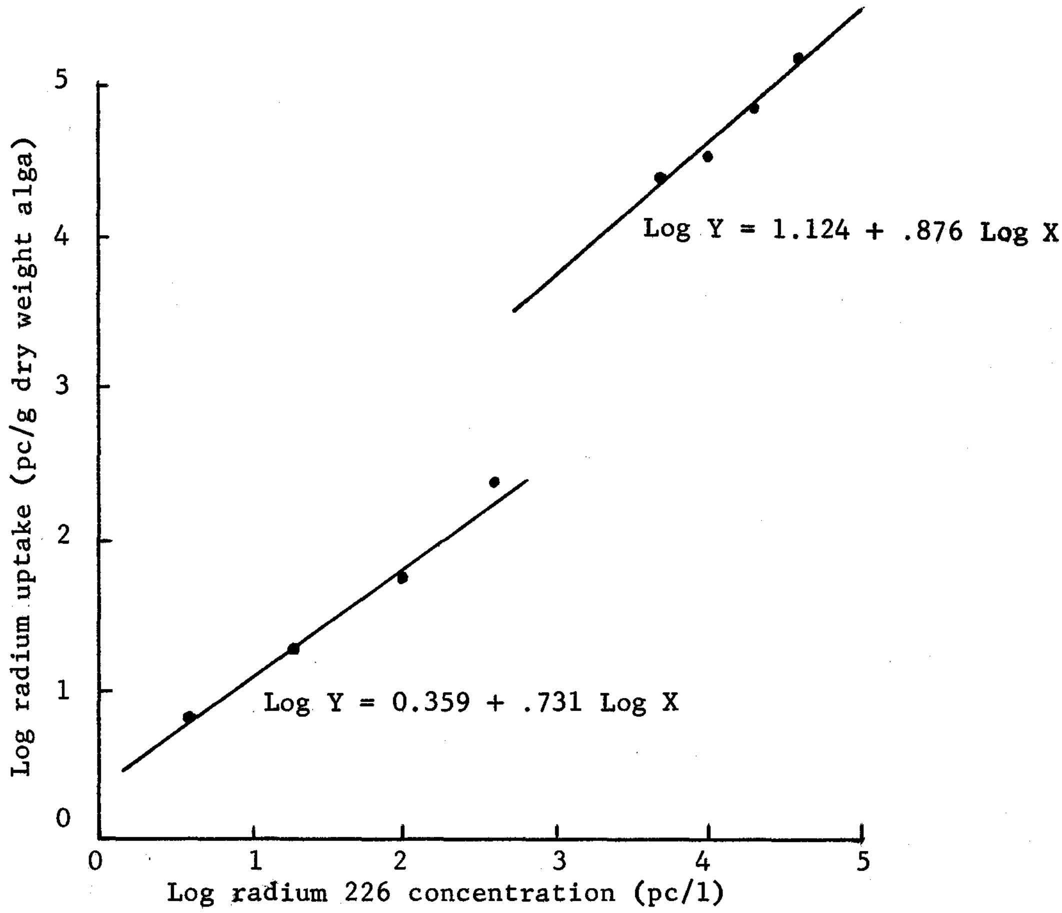

Figure 24. Relationship of radium uptake by <u>Vaucheria</u> to the concentration of radium in the medium. Two experiments using different radium concentrations are shown.

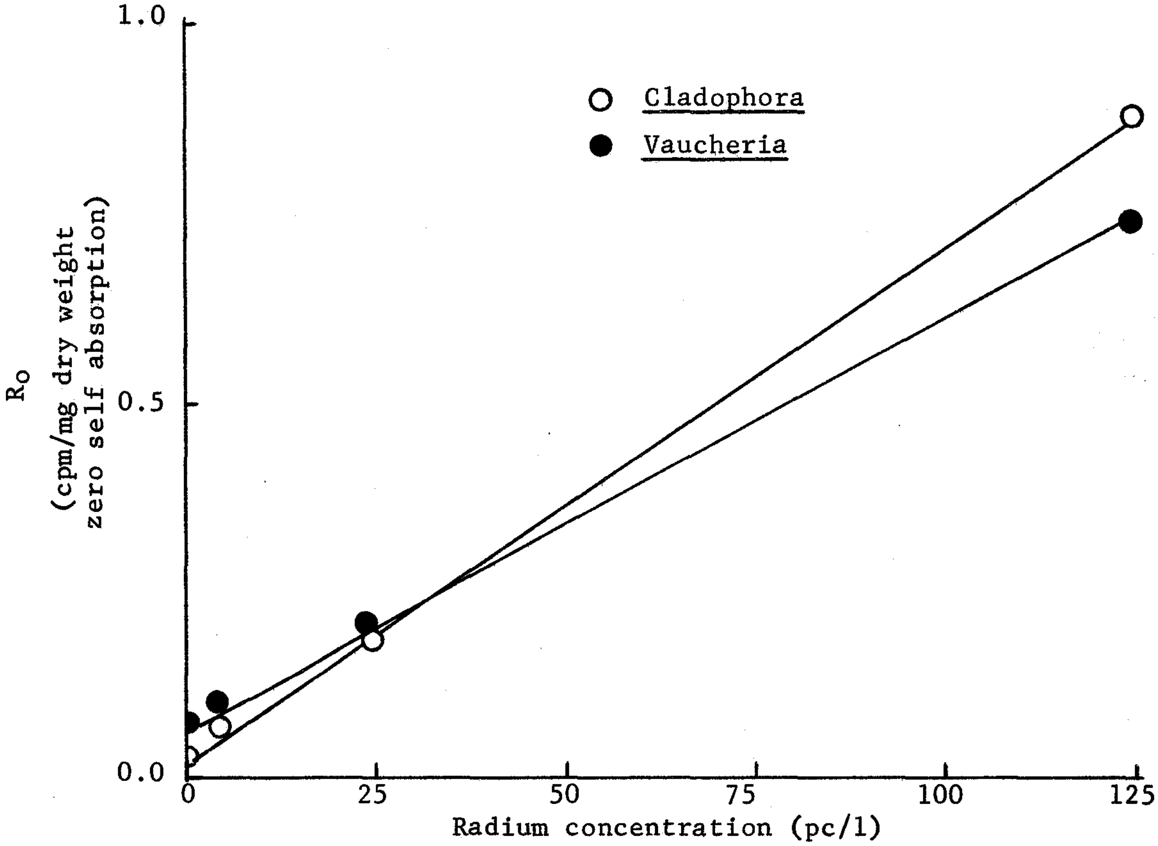

Figure 25. Relationship of radium 226 uptake by *Cladophora* and *Vaucheria* to the concentration of radium in the medium

The adsorption that occurred between radium and algae appeared to be polar rather than mechanical. If it had been mechanical, that is, bound with weak residual and secondary valences, the radium would have been removed during washing. It seemed likely that the radium combined with an organic acid to form a salt. Wasserman (1949) and Eppley and Blinks (1957) found that acidic polysaccharides such as alginic acid, sulfated galactans, and polyuronides in cell walls acted as cation absorbers.

The concentration factors reported for the algae, although higher than other reported values, were low estimates. Because the radium in the medium was not maintained at a constant level, it decreased throughout the experiment. No equilibrium constant was established for radium in the medium, and since concentration factors are based on a ratio of the amount in the algae to the amount in the medium, the reported concentration factors had to be based on the original amount of radium in the medium. This obviously gave much lower concentration factors than actually occurred. The fact that concentration factors were highest in the lowest radium concentrations was because the amounts of radium accumulated by the algae were not in the same proportions as the differences between radium concentrations. Thus, ratios of radium in algae to radium in water were very high in the low radium concentrations. This seemed to contradict a previous statement that radium concentrations were never great enough to fill all available adsorption sites and so it was concluded that adsorption was not the only process involved in radium accumulation. All three major processes probably played some part in radium uptake although no definite proof was established.

The results of radium uptake experiments all indicated a decrease in radium at 48 hours. This was explained, based on the assumption that radium reacts similarly to calcium, as possibly being due to an interaction between radium and potassium ions. It had been shown previously that uptake of alkaline earth cations are commonly depressed by the addition of alkali cations. Overstreet (1952) suggested a competition between calcium and potassium for absorption sites when calcium absorption by barley roots fell rapidly as potassium concentrations were increased. Table 22 indicated a considerable rise in potassium concentration of the medium at 48 hours, which, if competition for site existed, could account for the decrease in radium. It was supposed this increased potassium came from algal cells that had been injured or killed when they were placed in the macrocosms. Another possible explanation was that radium uptake by algae was dependent upon the ionic composition of the medium, as Austin (1963) found for strontium uptake by *Chlorella*. He noted that a strong influence was exerted on strontium uptake by the divalent cations calcium and magnesium. Table 22 showed that at 24 hours, when radium uptake was the greatest, the calcium and magnesium concentrations of the solution were lowest. When the idea of ion competition was carried one step further and the total calcium, magnesium, and potassium content of the medium were looked at, their combined concentrations were highest when radium uptake was lowest.

Algal production

Production of the algae tested was affected by the radium in the media. Some measurements indicated production was inhibited while

Table 22. Chemical analyses of water in the macrocosms at the four sampling periods

Time hr.	TDS %	Calcium ppm	Magnesium ppm	Sodium ppm	Potassium ppm	Chloride ppm	Sulfate ppm	Carbonate ppm	Bicarbonate ppm
12	.0204	48	14	3	1	0	6	0	215
24	.0198	43	13	3	4	2	11	0	207
48	.0200	42	17	4	10	0	10	0	235
96	.0198	49	17	3	4	0	8	0	246

others showed it was stimulated and the effects of radium on production varied according to the algae. Krauss (1962) noted that the inhibitory action of radioactive compounds had been known but little had been done to identify the precise mechanisms. Stimulation of processes in plants had been attributed to radiations from radium for years (Gager, 1936).

Respiration in both Vaucheria and Cladophora increased as the concentration of radium was raised (Figure 12, 13). Respiration was greater than in the controls in both algae, when the radium concentration reached 125 pc/l. This was in agreement with Gager (1936) who reported an extraordinary increase in the respiration of cells exposed to alpha emanations of radium and other investigators who had indicated an increased respiration. Kandler and Sironval (1959) determined that very high light intensities had the same effect on algae. They found an increase of 250 percent in the endogenous respiration. Stoklass (1926)[5] explained increased respiration was caused by the stimulation of oxidase and peroxidase which form the oxidation products that are finally split into carbon dioxide and hydrogen.

In addition to the radiation there was the possibility of increased respiration due to the addition of the radium chloride. Bennet-Clark and Bexon (1943) and Robertson (1941) reported an increase in respiration after addition of chlorides of calcium and magnesium to the medium. They assumed the increased respiration was due to the interchange of cations between tissue and medium and an accumulation of salt in the tissues. Both of these processes seemed to occur in these experiments and may have played a part in the increased respiration.

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[5]From Gager, C.S. 1936. The effect of radium rays on plants. In Biological Effects of Radiation, Vol. II, Chapt. 30, pp. 987-1013, B. M. Duggar, ed. McGraw-Hill Book Co., Inc., N.Y.

Photosynthesis, in terms of oxygen production, was not as sensitive to radiation as was respiration. Though both demonstrated similar reactions to radium, net oxygen production did not show as large a response to radium as respiration. In both algae the net oxygen production became negative as the concentration of radium was raised; however, Cladophora maintained a positive net oxygen production in radium concentrations as high as 25 pc/l while net oxygen production in Vaucheria became negative when the concentrations of radium got higher than 5 pc/l. In both algae, under the experimental conditions, the control algae and algae in the lowest concentration of radium had a positive oxygen response for at least 24 hours while algae in the higher concentrations of radium never maintained a positive production for longer than 12 hours. In general, oxygen production appeared to be inhibited by the experimental conditions. Inhibition of photosynthetic ability by alpha radiation was reported previously by Rabinowitch (1945). Gailey and Tolbert (1958) thought this inhibition of photosynthesis was a consequence of a decline of chlorophyll formation as a result of change in enzyme synthesis.

The stimulation of respiration at radiation doses that inhibited photosynthesis seemed to indicate the two processes are controlled by separate mechanisms with different radiation sensitivities.

The effects of radiation on production, when it is measured as carbon fixation, showed the same type of results. Data demonstrated a decided difference in algal sensitivities and there were indications of increased respiration at certain concentrations of radium.

Difference in the sensitivities of the two filamentous algae was indicated in Figure 18. Under the experimental conditions *Vaucheria* continued to increase its carbon uptake while *Cladophora* carbon uptake decreased. This difference in sensitivity was shown by the oxygen measurements also (Figure 12, 13). This was not surprising since in their metabolism and organization algae often differ as much from each other as they do from other groups of organisms. Because of this, a great variation between responses was not unexpected. Due to this variation, it was difficult to make generalizations concerning the mode of reaction to radium.

There was a decrease in carbon uptake by algae in the radium concentrations approximating the concentrations of radium at which respiration was observed to increase previously (Figure 17). This suggested the possibility that the net carbon 14 uptake in some of the alga was less. It appeared the alga in all concentrations of radium had approximately the same rate of total carbon fixation but the alga in 50, 150, and 450 pc/l had an increased rate of respiration.

As the radium concentrations became higher the rate of respiration was inhibited as is the case with many agents that cause stimulation when applied in small amounts. The data from both the oxygen production and carbon 14 uptake investigations agreed with this. Another possible explanation was that the intermediate radium concentrations inhibited the carbon fixation mechanism though it was unaffected by the highest concentrations. This did not seem feasible.

In long-term exposure to radium, the continued exposure had more effect on the productivity of algae than the concentration of radium did. In the investigations of *Chlorella* productivity, which ran for 40 days, the length of exposure affected all the measurements taken.

Total nitrogen, lipids, and dry weights were taken as production measurements on the Chlorella in addition to oxygen production and carbon 14 uptake. Theoretically, primary production can be measured by one of the principal molecular constituents, such as protein, lipid, or carbohydrate or by actual growth. Since it was not known what type of effect long term exposure might have, these additional measurements were included.

A good relationship existed between the effects of radium on carbon 14 uptake and net oxygen production in Chlorella (Figure 22). Here, as in filamentous algae, the decrease in both processes implied an increased respiration rate. The interaction surface of the oxygen response showed that after 10 days the net production dropped to a level that was about the same in all concentrations of radium (Figure 16).

The drop in oxygen production after 10 days was thought at first to be due to an inhibition of the photosynthetic mechanism. It had been reported by Redford and Myers (1951) and Kandler (1958) that the photosynthetic mechanism was not as sensitive to radiation as the respiratory mechanism and required a longer time to show an effect. This decreased photosynthetic rate, however, was merely a reflection of the age and decreased growth of the Chlorella culture. Examination of the data on dry weights indicated a reduction in growth rate which was reflected in a reduction of photosynthesis (Table 23, Figure 26). In addition, there was a drop in net oxygen production of the controls that was very similar to the reductions in the treatment levels. The only indication of increased respiration was demonstrated at 10 days in

Table 23. Mean values of dry weight of Chlorella in grams per milliliter as affected by concentration of radium 226 and length of exposure

Conc. Ra 226 (pc/l)	Time** (days)				Total
	10	20	30	40	
0	.0501	.1536	.1634	.1898	.5569
6	.0280	.1483	.2255	.3132	.7150
30	.0391	.1418	.1765	.2061	.5635
150	.0671	.1586	.1790	.2118	.6165
1350	.0240	.1494	.1529	.2066	.5329
Total	.2083	.7517	.8973	1.1275	

**Significant at 0.5 percent probability

30 pc/l. The interaction surface of the carbon 14 followed a similar pattern except for an apparent recovery at 40 days (Table 20, Figure 21).

The interaction surface formed by the total nitrogen response demonstrated a decrease of nitrogen followed by a recovery after 40 days. There was stimulation of nitrogen assimilation in all the algae subjecte to radium (Table 24, Figure 27). The total nitrogen content of the algae ranged from less than 1 to better than 3 percent of the dry weight and agreed well with values reported in the literature. In unicellular algae approximately 80 percent of this nitrogen represents protein (Fowden, 1962). This protein in algae is thought to be predominantly enzymes with specific biological roles. Since the total nitrogen measurements represent mainly protein, and if most of the proteins are enzymes, the data from these experiments with radium conflicted with that reported

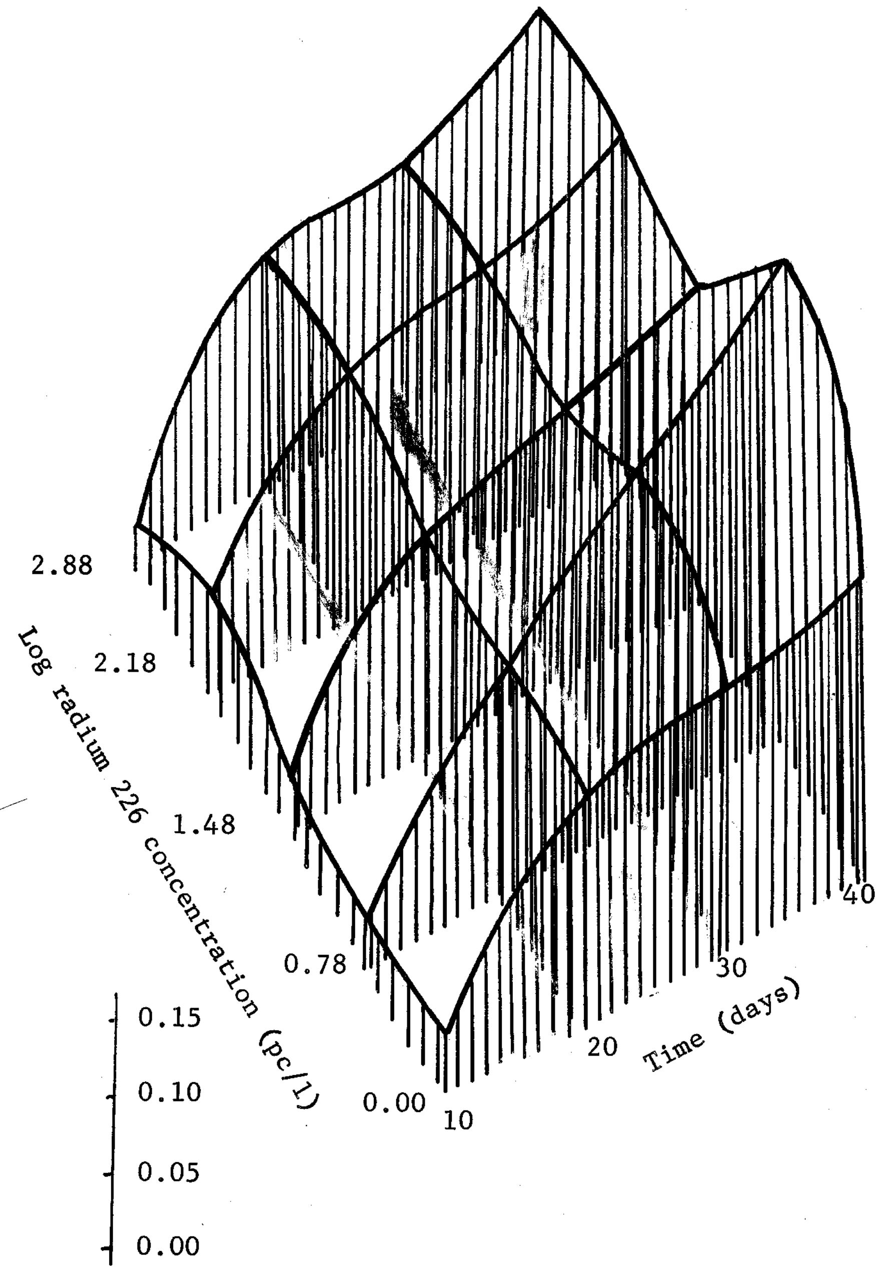

Figure 26. Exploration of the interaction surface formed by the growth response of Chlorella exposed to five treatment levels of radium for four time periods.

Table 24. Mean values of total nitrogen content of Chlorella in milligrams per gram dry weight as affected by concentration of radium 226 and length of exposure

Conc.* Ra 226 (pc/1)	Time** (days) 10	20	30	40	Total
0	25.615	14.759	10.172	11.457	62.003
6	27.952	11.991	27.249	21.770	88.962
30	33.043	7.776	20.362	23.165	84.346
150	27.539	13.894	11.152	27.284	79.869
750	24.567	8.578	13.687	20.583	67.415
Total	138.716	56.998	82.622	104.259	

**Significant at 0.5 percent probability.
*Significant at 10.0 percent probability

by Kuzin (1956). Kuzin indicated denaturation and precipitation occurred in proteins exposed to radium salts. He also reported the denaturation of amino acids and inactivation of enzymes exposed to alpha radiations. If either denaturation of proteins or inactivation of enzymes had occurred in this investigation, there would have been no stimulation of total nitrogen content. Stimulation such as that observed here was reported by Stoklass (1926)[6] for enzymatic processes exposed to alpha radiation.

The interaction of the lipid response demonstrated no difference in response due to radium concentrations and only a slight increase followed by a decrease through time (Table 25, Figure 28. This agreed with other reported data. Kuzin (1956) stated there were few effects

[6]From Gager (1936).

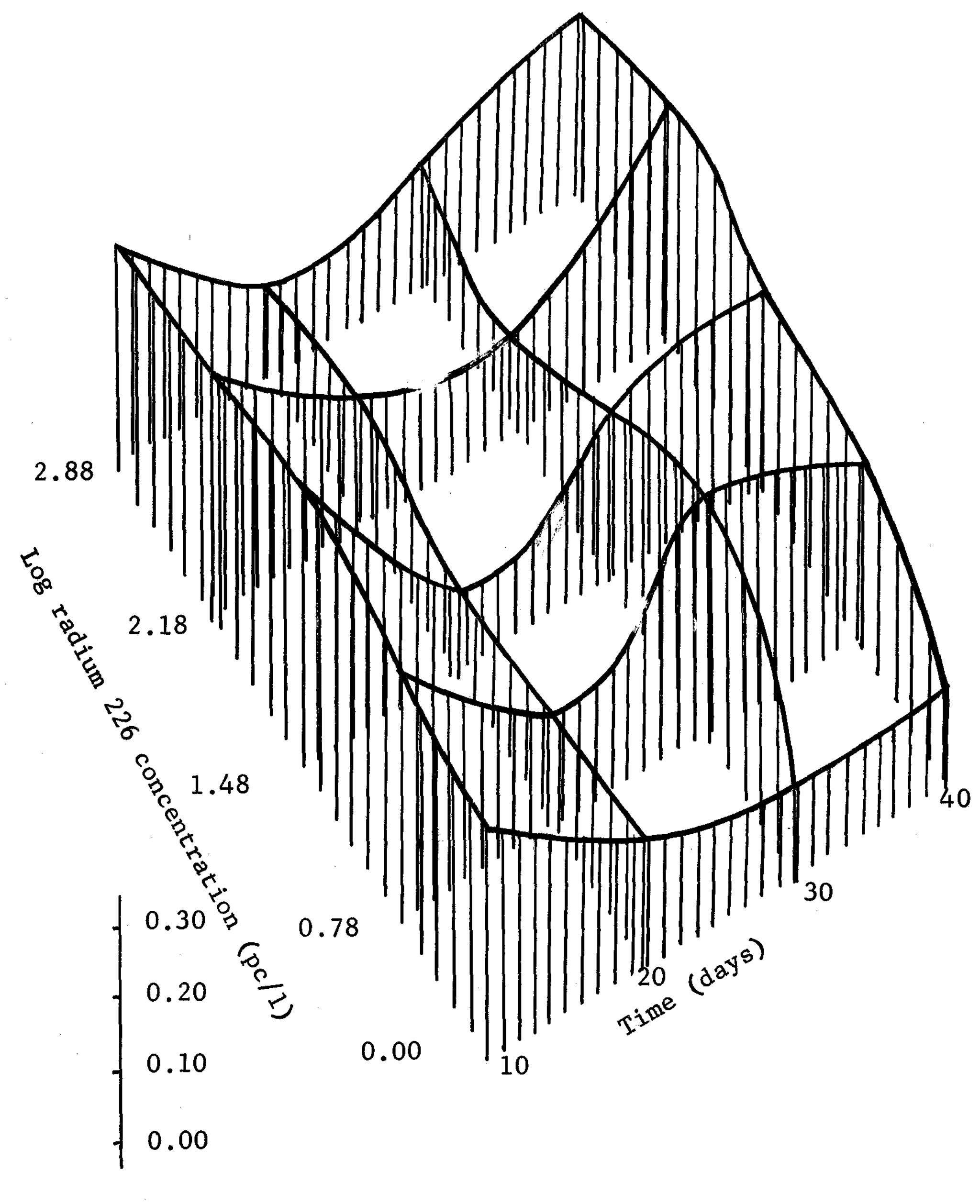

Figure 27. Exploration of the interaction surface formed by the total nitrogen response of Chlorella exposed to five treatment levels of radium for four time periods.

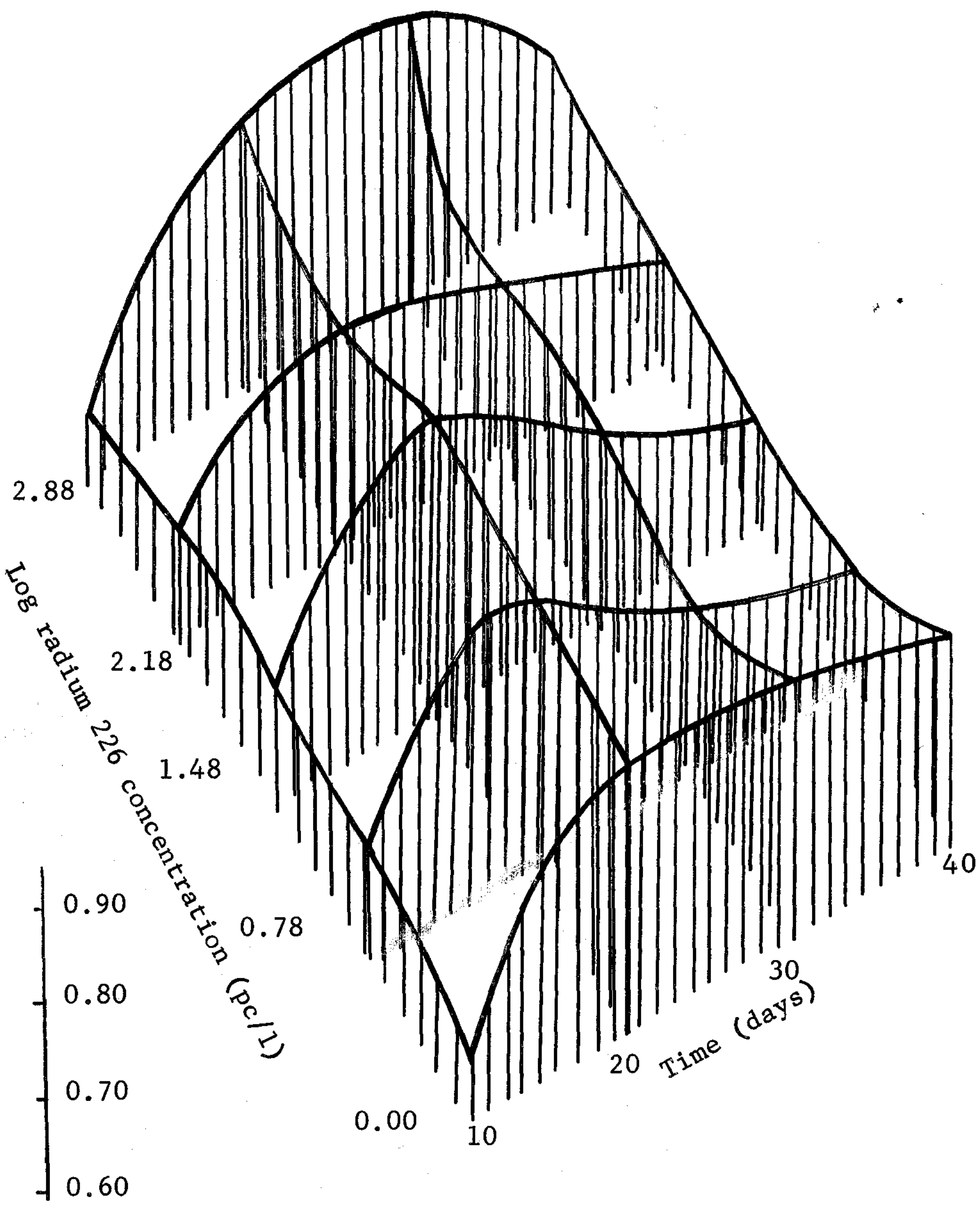

Figure 28. Exploration of the interaction surface formed by the total lipid response of Chlorella exposed to five treatment levels of radium for four time periods.

Table 25. Mean values of the lipid content of Chlorella in milligrams per gram dry weight as affected by concentration of radium 226 and length of exposure

Conc. Ra 226 (pc/l)	Time** (days) 10	20	30	40	Total
0	.6741	.8820	.8219	.8231	3.2011
6	.7325	.9005	.7715	.7181	3.1226
30	.7336	.9175	.7990	.7166	3.1667
150	.7306	.8413	.7809	.7051	3.0579
1350	.6792	.8975	.9088	.7319	3.2174
Total	3.5500	4.4388	4.0821	3.6948	

**Significant at 0.5 percent probability.

of radiation on lipids unless the radiation dose was extremely high. The lipid data also agreed well with the total nitrogen data. The growth rate of algae depends upon the protein content of the cells rather than the lipid content and the accumulation of lipids begins when growth ceases. In the Chlorella the lipid content increased when the total nitrogen content decreased and indicated a slowing down of the growth rate at these points.

When Chlorella production was measured as dry weight the responses showed that after 20 days the production of algae exposed to radium exceeded that of the controls (Table 23, Figure 26). The Chlorella grown in the lowest concentration of radium demonstrated the best production. It appeared that the only cultures whose growth had begun to slow down to any extent were the controls.

Correlation coefficients indicated a significant relationship between four of the productivity measurements: total nitrogen, lipids, carbon uptake, and oxygen production (Table 21). Radium uptake, on the other hand, was significantly related to only the dry weight of algae. This seemed to imply that perhaps radium uptake in Chlorella was not connected with any active metabolic process. The four related measurements were all processes that require a metabolically active system. Dry weight, however, could be interpreted as a measurement of surfaces available for sorption. From this it was concluded that sorption was the most important factor in the accumulation of radium by Chlorella.

Results of the food chain experiment indicated radium was present in all the trophic levels investigated in greater quantities than was expected. It was assumed this was due to radium uptake by the organisms through adsorption as well as ingestion. Hoffman and Olive (1961) reported the uptake of phosphorus 32 by trophic levels above primary producers was proportional to the surface area available. No matter what the mechanism of uptake, the fact remained that organisms in the food chain were exposed to more radium than had been anticipated and the possibilities of both health hazards and changes in the ecosystem were enhanced.

SUMMARY AND CONCLUSIONS

Summary

Three genera of algae were cultured in media containing different amounts of dissolved radium. The experimental conditions were controlled so that the uptake and accumulation of radium and its effects on algal productivity could be studied. Each experiment was designed for statistical analysis and contained five treatment levels of radium, four to six replications of each level, and four time periods. The ultimate objective of the investigation was to enable meaningful predictions to be made of possible results to man or the aquatic ecosystem from radium contamination.

After exposure to radium for a predetermined length of time, the algae were analyzed either for radium content or changes in productivity. Radium analyses showed the algae accumulated radium to varying degrees. Patterns of uptake and concentration varied between algae and statistical tests confirmed that the differences were real.

The measurements used to determine changes in productivity were carbon fixation, respiration, oxygen production, dry weight, total nitrogen, and total lipids. Each of these production characteristics demonstrated some change. Response patterns were extremely variable but it was shown statistically that either the amount of radium or the length of exposure affected each parameter.

In low concentrations radium sometimes accelerated certain of the metabolic processes while it retarded others, but in higher

concentrations all activities were depressed. Carbon fixation was decreased as the radium concentrations increased from the controls to 160 pc/l, respiration was increased in concentrations between 25 and 125 pc/l, while oxygen production was inhibited in all concentrations of radium. Algal growth was stimulated at 6 pc/l while the nitrogen content of algae was higher in all concentrations of radium than in the controls.

From these experiments it appeared that radium may either stimulate, inhibit, or have no effect on productivity measurements and the response from a particular measurement can vary between algae.

Conclusions

Investigations of radium uptake by algae show definitely that algae do accumulate radium and to a greater extent than had been suspected. The fact that there is a large variation between amounts of radium accumulated during different experiments strongly indicates that the extent of radium accumulation is dependent upon the type of algae, its physiological condition, and the physical and chemical characteristics of the environment. Further, since algae continue to accumulate radium for a considerable length of time, since their rate of radium uptake is not constant, and they accumulate radium against a concentration gradient, it is concluded that radium accumulation by algae is a multiple process probably involving adsorption, absorption, and metabolism.

While results of the investigation demonstrate there are effects on the algae caused by exposure to radium, it is impossible to say whether the effects of radium are due to its radioactive or chemical properties.

Either or both could have an effect. If a stable isotope of radium existed it would be possible to determine which causes the observed effects. Since it is not known which causes the effects and because of the variation in the sensitivities of organisms to different types of ionizing radiation, generalizations as to the ecological implications of radioactive contamination are impossible from the results of this study.

Data from this research are not adequate to permit exact predictions of the over-all effect of radium contamination on an aquatic ecosystem. However, results strongly indicate that its effect on the production of an ecosystem will be detrimental. Certain measurements show that algal production is stimulated by small concentrations of radium but, at concentrations higher than this optimum, all the measurements indicate that algal production is inhibited. Since algae tend to concentrate radium, any benefit that might occur due to stimulation from low concentrations of radium will be negated when the accumulated radium reaches an inhibitory level. This inhibition of the primary producers will reduce the production of the entire ecosystem.

The ability of algae to concentrate radium makes them a potential reservoir of this radionuclide for the entire ecosystem. It appears from the data that there is considerable conservation of radium as it passes from one trophic level to the next and, should it reach the apex of the food pyramid, the concentration factors of the algae imply a serious health hazard. This is especially true since radium 226 is a bone seeker with about a 50 year biological half-life. This long retention time and the high relative biological effectiveness of alpha emissions make it particularly dangerous. In addition to being a direct hazard to man, there is also the possibility of a change in the aquatic ecosystem if any organism or trophic level is sensitive to radium.

Because of the need for knowledge of any potentially dangerous contaminant, further work on the effects of radium are needed. Especially valuable would be research on the passage of radium through the food chain, the effects of different environmental conditions on its passage, and the effects of radium on the various trophic levels.

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APPENDIXES

Appendix A

Statistical analyses of experiments

Table 26. Analysis of variance of radium uptake by *Vaucheria* in cpm per milligram ash weight as affected by concentration of radium 226 and length of exposure. The concentration range of radium was from 0 - 40,000 pc/l

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	159	37,296.94		
Replications	3	981.92	327.31	1.19
Time	3	9,413.67	3,137.89	11.36**
$Time_L$	1	649.21	649.21	2.35
$Time_Q$	1	2,368.60	2,368.60	8.57**
$Time_C$	1	6,395.86	6,395.86	23.16**
Rep. x Time	9	4,981.90	553.54	2.00
Concentration	4	5,917.48	1,479.37	5.35**
$Conc_L$	1	5,402.02	5,402.02	19.56**
$Conc_Q$	1	459.12	459.12	1.66
$Conc_C$	1	77.46	77.46	0.28
Conc. x Time	12	1,639.45	636.62	2.30*
Error	48	13,251.72	276.07	
Determination	80	92.71	1.16	0.00

**Significant at 0.5 percent probability.
*Significant at 5.0 percent probability.

Table 27. Analysis of variance of radium uptake by Vaucheria in cpm per milligram ash weight. These are the same data used in Table 28 but tanks and replications are lumped

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	119	7,217.74		
Replications	3	40.15	13.38	0.34
Time	2	1,531.87	765.94	19.52**
$Time_L$	1	1,223.83	1,223.83	31.20**
$Time_Q$	1	308.04	308.04	7.85**
Concentration	4	2,520.61	630.15	16.06**
$Conc_L$	1	1,456.77	1,456.77	37.13**
$Conc_Q$	1	259.27	259.27	6.61*
Conc x Time	8	1,458.16	182.27	4.65**
Error	42	1,647.86	39.23	
Determinations	60	19.09	0.32	0.01

** Significant at 0.5 percent probabiliy
* Significant at 5.0 percent probability

Table 28. Analysis of variance of radium uptake by Vaucheria in cpm per milligram ash weight as affected by concentration of radium 226 length of exposure, and tank. The concentrations of radium were between 0 and 125 pc/1

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	119	7,217.74		
Replications	1	24.42	24.42	0.53
Tanks	1	0.58	0.58	0.01
Rep. x tanks	1	15.14	15.14	0.33
Time	2	1,531.87	765.94	16.50**
Rep. x Time	2	162.47	81.24	1.75
Concentration	4	2,520.61	630.15	13.57**
Tank x Time	2	9.18	4.59	0.10
Tank x Conc	4	63.59	15.90	0.34
Time x Conc	8	1,458.16	182.27	3.93**
Tank x Time x Conc	8	205.52	25.69	0.55
Error	26	1,207.11	46.43	
Determinations	60	19.09	0.32	0.01

**Significant at 0.5 percent probability.

Table 29. Analysis of variance of radium uptake by _Cladophora_ and _Vaucheria_ in cpm per milligram ash weight as affected by concentration of radium and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	239	14.5824		
Replications	5	0.1638	0.0328	5.05
Algae	1	0.5677	0.05677	87.34**
Error (a)	5	0.0327	0.0065	
Time	3	0.3187	0.1062	5.53**
Algae x Time	3	1.0042	0.3347	17.43**
Concentration	4	7.1694	1.7923	93.35**
Conc x Algae	4	1.5193	0.3798	19.78**
Conc x Time	12	.5520	0.0460	2.40**
Algae x Time x Conc	12	.4841	0.0403	2.10
Error (b)	190	3.6567	0.0192	

** Significant at 0.5 percent probability

Table 30. Analysis of variance of radium uptake by Chlorella as cpm per milligram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	39	463.994		
Replications	1	0.062	0.062	0.02
Time	1	117.669	39.223	12.12**
$Time_L$	1	100.392	100.392	31.01**
$Time_Q$	1	1.501	1.501	0.46
Concentration	4	77.994	19.499	6.02**
$Conc_L$	1	39.141	39.141	12.09**
$Conc_Q$	1	1.769	1.769	0.55
Time x Conc	12	206.766	17.231	5.32**
Error	19	61.503	3.237	

** Significant at 0.5 percent probability

Table 31. Analysis of variance of gross production by Vaucheria in ppm oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	239	14.6303		
Replications	5	0.2194	0.0439	0.84
Time	3	0.9148	0.3049	5.85***
$Time_L$	1	0.5408	0.5408	10.38***
$Time_Q$	1	0.3716	0.3716	7.13**
$Time_C$	1	0.0024	0.0024	0.05
Concentration	4	0.4109	0.1027	1.97*
$Conc_L$	1	0.0132	0.0132	0.25
$Conc_Q$	1	0.3297	0.3297	6.33**
$Conc_C$	1	0.0045	0.0045	0.09
Time x Conc	12	1.8856	0.1571	3.02***
Error	215	11.1996	0.0521	
Determinations	120	1.2188	0.0102	0.19

*** Significant at 0.5 percent probability
** Significant at 2.5 percent probability
* Significant at 10.0 percent probability

Table 32. Analysis of variance of respiration by Vaucheria in ppm of oxygen consumed per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	239	10.4747		
Replications	5	0.9143	0.1829	6.01**
Time	3	1.4557	0.4852	15.96**
$Time_L$	1	0.0241	0.0241	0.79
$Time_Q$	1	1.4182	1.4182	46.65**
$Time_C$	1	0.0133	0.0133	0.44
Concentration	4	0.3816	0.0954	3.14*
$Conc_L$	1	0.0380	0.0380	1.25
$Conc_Q$	1	0.2825	0.2825	9.29**
$Conc_C$	1	0.0016	0.0016	0.05
Time x Conc	12	1.1871	0.0989	3.25**
Error	215	6.5360	0.0304	
Determinations	120	0.8399	0.0070	0.23

** Significant at 0.5 percent probability
 * Significant at 2.5 percent probability

Table 33. Analysis of variance of net production by <u>Vaucheria</u> in ppm oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	239	8.1422		
Replications	5	1.1739	0.2348	8.85**
Time	3	0.5843	0.1948	7.35**
$Time_L$	1	0.3331	0.3331	12.57**
$Time_Q$	1	0.1744	0.1744	6.58*
$Time_C$	1	0.0151	0.0151	0.57
Concentration	4	0.0345	0.0086	0.35
Time x Conc	12	0.6491	0.0541	2.04*
Error	215	5.7005	0.0265	
Determinations	120	0.7912	0.0066	0.25

** Significant at 0.5 percent probability
* Significant at 2.5 percent probability

Table 34. Analysis of variance of gross production by Cladophora in ppm oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	239	83.8221		
Replications	5	3.9638	0.7928	6.81**
Time	3	50.2952	16.7651	114.03**
$Time_L$	1	24.5251	24.5251	210.70**
$Time_Q$	1	14.7535	14.7535	126.75**
$Time_C$	1	11.0166	11.0166	94.64**
Concentration	4	0.2974	0.0744	0.64
Time x Conc	12	1.9472	0.1623	1.39
Error	215	25.0195	0.1164	
Determinations	120	2.2989	0.0192	0.17

** Significant at 0.5 percent probability

Table 35. Analysis of variance of respiration by Cladophora in ppm of oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	239	16.7228		
Replications	5	0.8121	0.1624	3.97**
Time	3	4.6116	1.5372	37.58**
$Time_L$	1	1.7770	1.7770	43.45**
$Time_Q$	1	2.4663	2.4663	60.30**
$Time_C$	1	0.3683	0.3683	9.00**
Concentrations	4	0.7051	0.1763	4.31**
$Conc_L$	1	0.5249	0.5249	12.83**
$Conc_Q$	1	0.0266	0.0266	0.65
$Conc_C$	1	0.1535	0.1535	3.75
Time x Conc	12	1.0239	0.0853	2.09*
Error	215	8.7852	0.0409	
Determinations	120	0.7848	0.0065	0.16

** Significant at 0.5 percent probability
* Significant at 2.5 percent probability

Table 36. Analysis of variance of net production by Cladophora in ppm oxygen per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	239	44.3566		
Replications	5	2.8514	0.5703	9.49**
Time	3	25.6129	8.5376	142.06**
$Time_L$	1	13.1117	13.1117	218.16**
$Time_Q$	1	5.1740	5.1740	86.09**
$Time_C$	1	7.3271	7.3271	121.92**
Concentration	4	0.6222	0.1556	2.59*
$Conc_L$	1	0.1074	0.1074	1.79
$Conc_Q$	1	0.0001	0.0001	0.00
$Conc_C$	1	0.5068	0.5068	8.43**
Time x Conc	12	1.2888	0.1074	1.54
Error	215	12.9116	0.0601	
Determinations	120	1.0698	0.0089	0.15

** Significant at 0.5 percent probability
 * Significant at 5.0 percent probability

Table 37. Analysis of variance of net production by Chlorella in ppm oxygen per milligram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	39	1.8514		
Replications	1	0.0404	0.0404	1.25
Time	3	1.0793	0.3598	11.17*
$Time_L$	1	0.6977	0.977	21.67*
$Time_Q$	1	0.3506	0.3506	10.89*
Error (a)	3	0.0966	0.0322	
Concentration	4	0.1693	0.0423	6.82**
$Conc_L$	1	0.0661	0.0661	10.66**
$Conc_Q$	1	0.0129	0.0129	2.08
Time x Conc	12	0.3652	0.0304	4.90**
Error (b)	16	0.1006	0.0062	

** Significant at 1.0 percent probability
 * Significant at 5.0 percent probability

Table 38. Analysis of variance of carbon 14 uptake by Vaucheria and Cladophora in cpm per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	159	325,673.91		
Replications	3	1,570.19	523.39	1.11
Algae	1	56,925.51	56,925.51	121.22**
Error (a)	3	1,408.83	469.61	
Time	3	31,663.22	10,554.40	14.57**
$Time_L$	1	30,364.36	30,354.36	43.08**
$Time_Q$	1	15.17	15.17	0.02
Concentration	4	25,663.98	6,415.99	9.10**
$Conc_L$	1	16.15	16.15	0.02
$Conc_Q$	1	14,417.67	14,417.67	20.45**
Algae x Time	3	82,130.97	27,377.70	38.84**
Algae x Conc	4	5,486.73	1,371.68	1.95*
Time x Conc	12	27,149.68	2,262.47	3.21**
Algae x Time x Conc	12	13,316.05	1,109.67	1.57
Error (b)	114	80,358	704.90	

** Significant at 0.5 percent probability
* Significant at 5.0 percent probability

Table 39. Analysis of variance of carbon 14 uptake by Vaucheria in cpm per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	79	75,311.44		
Replications	3	2,687.13	895.71	1.38
Time	3	6,230.23	2,076.21	3.21**
$Time_L$	1	6,177.81	6,177.81	9.54**
$Time_Q$	1	0.03	0.03	0.00
Concentration	4	14,848.68	3,712.17	5.73**
$Conc_L$	1	173.45	173.45	0.27
$Conc_Q$	1	8,757.86	8,757.86	13.52**
Time x Conc	12	14,628.66	1,219.06	1.88
Error	57	36,196.74	647.66	

** Significant at 1.0 percent probability
* Significant at 10.0 percent probability

Table 40. Analysis of variance of carbon 14 uptake by Cladophora in cpm per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	79	193,439.70		
Replications	3	296.64	98.21	0.13
Time	3	107,563.85	35,854.82	47.05**
$Time_L$	1	105,645.48	105,645.48	138.63**
$Time_Q$	1	32.16	32.16	0.04
Concentration	4	16,303.28	4,075.82	5.35**
$Conc_L$	1	355.52	355.52	0.47
$Conc_Q$	1	5,809.93	5,809.93	7.62**
Time x Conc	12	25,838.72	2,153.23	2.83**
Error	57	43,439.21	762.09	

** Significant at 1.0 percent probability

Table 41. Analysis of variance of carbon 14 by Chlorella as cpm per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	19	31,937.78		
Time	3	27,413.89	9,137.96	27.88**
Time_L	1	7,707.43	7,707.43	1.96
Time_Q	1	19,621.35	19,621.35	4.99*
Concentration	4	591.07	147.77	0.45
Error	12	3,932.82	327.73	

** Significant at 0.5 percent probability
* Significant at 5.0 percent probability

Table 42. Analysis of variance of the total nitrogen content of Chlorella in milligrams per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	59	4,791.60		
Replications	2	6.73	3.36	0.14
Time	3	2,155.41	718.47	30.10****
$Time_L$	1	214.65	214.65	8.99**
$Time_Q$	1	1,602.16	1,602.16	67.12****
Error (a)	6	143.23	23.87	
Concentration	4	390.71	97.68	2.58*
$Conc_L$	1	0.23	0.23	0.01
$Conc_Q$	1	331.74	331.74	8.76***
Time x Conc	12	883.04	73.59	2.00*
Error (b)	32	1,212.48	37.89	

**** Significant at 0.5 percent probability
*** Significant at 1.0 percent probability
** Significant at 2.5 percent probability
* Significant at 10.0 percent probability

Table 43. Analysis of variance of the dry weight of *Chlorella* in grams per mililiter as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	59	.4100		
Replications	2	.0008	.0004	0.29
Time	3	.2747	.0916	65.43**
$Time_L$	1	.2529	.2529	108.61**
$Time_Q$	1	.0147	.0147	10.50*
Error (a)	6	.0085	.0014	
Concentration	4	.0159	.0040	1.54
Time x Conc	12	.0272	.0023	0.89
Error (b)	32	.0829	.0026	

** Significant at 0.5 percent probability
 * Significant at 5.0 percent probability

Table 44. Analysis of variance of the lipid content of Chlorella in milligrams per gram dry weight as affected by concentration of radium 226 and length of exposure

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
Total	59	.6719		
Replications	2	.0078	.0039	0.57
Time	3	.2888	.0963	13.96**
Time_L	1	.0001	.0001	0.01
Time_Q	1	.2442	.2442	35.39**
Error (a)	6	.0414	.0069	
Concentration	4	.0125	.0031	0.52
Time x Conc	12	.0727	.0061	1.02
Error (b)	32	.1947	.0060	

** Significant at 0.5 percent probability

Table 45. Test to determine if there is a significant difference between concentration factors of Cladophora and Vaucheria

Time (days)	Concentration factors: Cladophora	Vaucheria	Difference
1/2	88	133	-45
	76	191	-115
	79	146	-67
	104	87	17
1	93	901	-808
	112	623	-511
	152	210	-58
	173	181	-8
2	119	371	-252
	125	113	12
	96	128	-32
	124	169	-45
4	484	354	130
	332	101	231
	218	119	99
	199	67	132

$t = \frac{(\bar{d}-0)}{S_{\bar{D}}} = 1.283$

$\Sigma d = -1320$

$\Sigma d^2 = 1,101,624$

$\bar{d} = -82.50$

$S_d^2 = 66,181.60$

Tabular $t_{.05} = 2.131$

∴ No significant difference between concentration factors of Vaucheria and Cladophora.

Table 46. Groups tested for common slopes in Table 47

Group number	Radium 226 conc. (pc/l)	Length of exposure (hr)
33	5,000	all times[a]
34	10,000	all times
35	20,000	all times
36	40,000	all times
37	4 high[b]	12
38	4 high	24
39	4 high	48
40	4 high	96
42	1	all times
43	5	all times
44	25	all times
45	125	all times
46	4 low[c]	12
47	4 low	24
48	4 low	48
49	4 low	96
51	all conc.	12
52	all conc.	24
53	all conc.	48
54	all conc.	96

[a]All times includes 12, 24, 48, and 96 hours.

[b]High concentrations are 5,000, 10,000, 20,000, and 40,000.

[c]Low concentrations are 1, 5, 25, and 125.

Table 47. Analyses of covariance of common slopes from radium uptake by _Vaucheria_ in eight concentrations of radium and four lengths of exposure. Numbers are groups of Table 46 being tested

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
37, 46				
A[a]	1	12,903,06	12,903.06	24.61*
B	52	27,259.13	524.21	
38, 47				
A	1	633,382	633,382	22.59*
B	52	1,457,870	48,035	
39, 48				
A	1	6,256.54	6,256.54	11.81*
B	52	27,984.83	538.17	
40, 49				
A	1	9,321.86	9,321.86	14.32*
B	52	33,848.27	650.93	
37, 38, 39, 40				
A	3	458,423	152,807	11.85*
B	120	1,546,961	12,891	
46, 47, 48, 49				
A	3	0.0731	0.243	2.00
B	88	1.0696	0.021	

[a]A = Due to common slope
B = Due to individual slopes
*Significant difference between slopes

Table 47 (cont'd.)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
37, 38, 39, 40, 46, 47, 48, 49				
A	7	753,121	107,588	14.47*
B	208	1,546,962	7,437	
51, 52				
A	1	403,936	403,936	20.45*
B	108	2,132,811	19,748	
51, 53				
A	1	763.34	763.34	1.11
B	108	74,589.71	690.65	
51, 53, 53, 54				
A	3	608,317	202,772	19.81*
B	216	2,210,614	10,234	
33, 34, 35, 36				
A	3	86,150	28,716	1.17
B	120	2,948,440	24,570	
42, 43, 44, 45				
A	3	0.0054	0.0018	0.15
B	88	1.0211	0.0116	
33, 34, 35, 36, 42, 43, 44, 45				
A	7	143,008	20,286	1.43
B	208	2,948,441	14,175	

Appendix B

Methods used for determining quantities of radium, oxygen, and unicellular algae

Table 48. Raw data in counts of radioactivity per gram ash weight of algae exposed to two levels of radium for 96 hours[a]. Average values are used to construct Figure 29 where lines are fitted by eye.

Conc. Ra 226 (pc/1)	Ash weight (mg)	5 min. counts Replication 1	2	Average cpm-bkg
	50	333	347	67.8
1	100	441	437	87.6
	250	479	473	95.0
	50	1,073	1,075	214.6
25	100	1,255	1,213	246.6
	250	1,478	1,437	291.3

[a]These values are used to illustrate procedures as given by: Tsivoglou, E.C. 1961. Method for gross radioactivity analysis of environmental samples. Radiological Pollution Activities Unit, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. 15p.

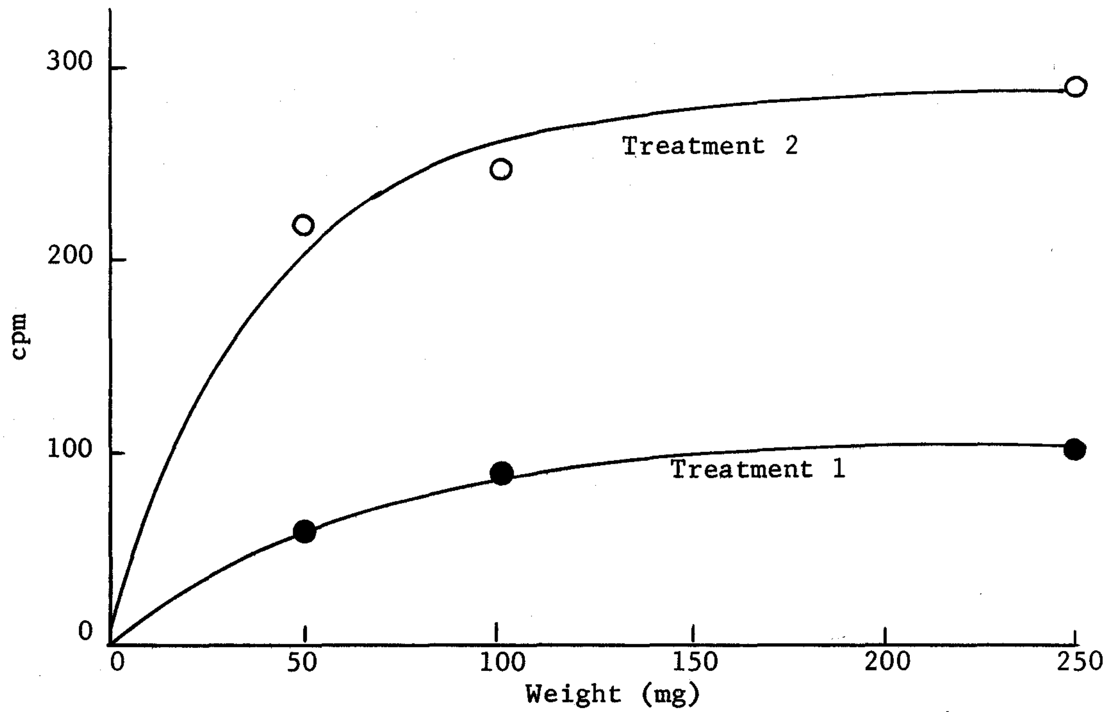

Figure 29. Plot of observed cpm versus ash weight of algae. Lines are fitted by eye.

Table 49. Changes in the count rate as given in Figure 29 by determining the difference in the cpm between each 50 milligram interval. The differences are plotted on semilog graph paper at the midpoint of the weight intervals and a line fitted by eye as in Figure 30[a]

Tr. 1 (I pc/1)					Tr. 2 (25 pc/1)				
wt. (mg)	cpm	Difference in cpm		Corrected cpm[b]	wt. (mg)	cpm	Difference in cpm		Corrected cpm
		Act	Corr.[c]				Act	Corr.	
0	0			0	0	0			0
50	68	68	62	62	50	203	203	201	201
100	86	18	20	82	100	261	58	65	266
150	92	6	6	88	150	283	22	21	287
200	94	2	2	90	200	290	7	7	294
250	95	1	1	91	250	292	2	2	296

[a]This step is to determine the count rate at zero self absorption.

[b]Based on the corrected changes in cpm. These corrections are a means of adjusting for errors in laboratory procedures. They are the figures used in the remaining calculations.

[c]Actual and corrected.

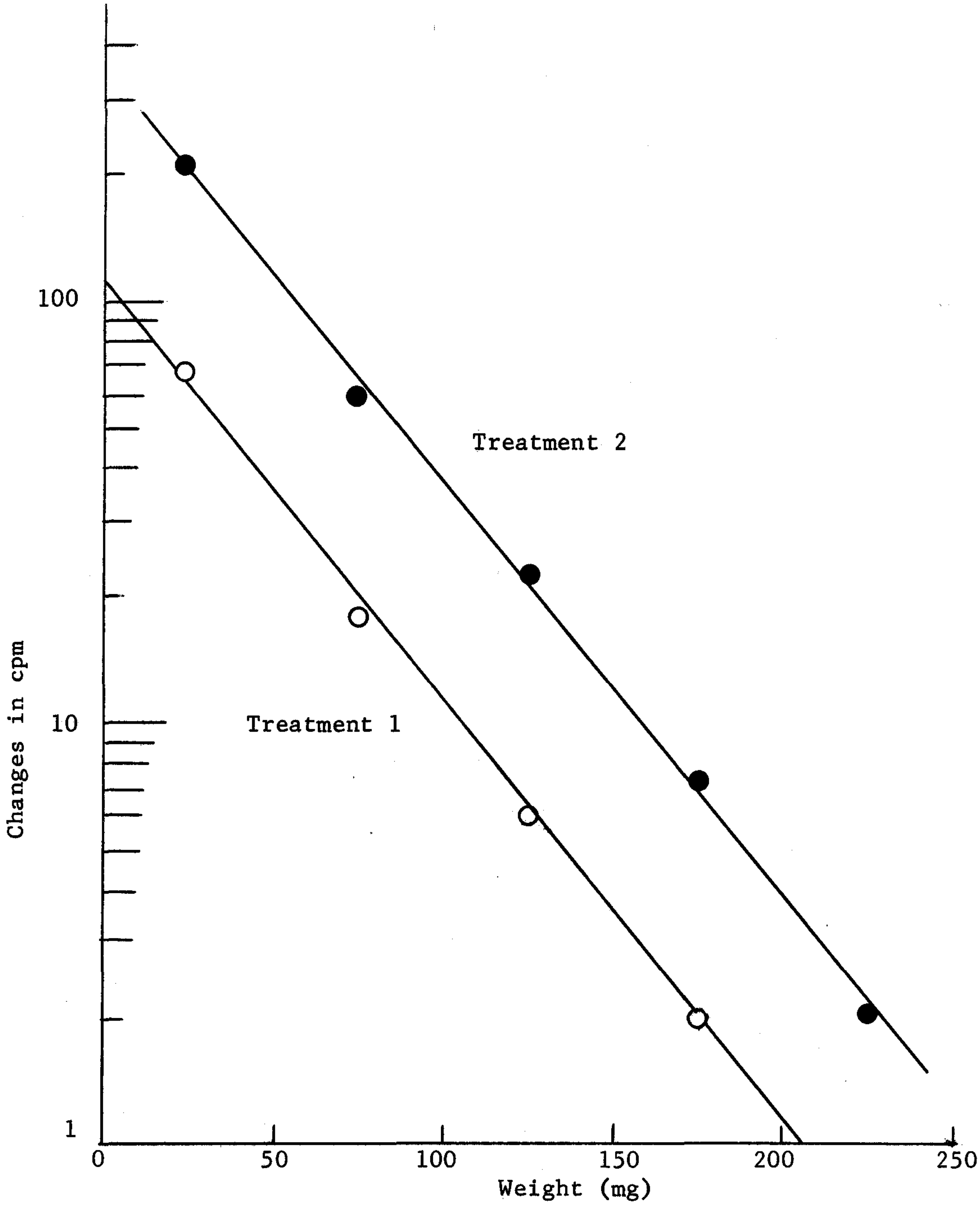

Figure 30. Semilog plot of the changes in cpm from Table 47. Lines are fitted by eye and corrected cpm are read from the lines.

Table 50. Calculations for converting from cpm to picocuries of radium per gram alga ash using corrected cpm from Table 49

Determination of C, the absorption coefficient, where:

$$C = \frac{\ln \text{(1st difference cpm/4th difference cpm)}}{\text{weight difference between 1st and 4th counts}}$$

$$\text{Tr } 1 = \frac{\ln 62/2}{150} = \frac{\ln 31}{150} = \frac{3.34}{150} = 0.0223 = C_1$$

$$\text{Tr } 2 = \frac{\ln 203/7}{150} = \frac{\ln 29}{150} = \frac{3.37}{150} = 0.0225 - C_2$$

Determination of r_o, zero thickness count rate, in cpm/mg where:

$$r_o = \frac{CRw}{1-e^{-cw}}$$ where C = absorption coefficient
R_w= corrected cpm at weight w

$$\text{Tr } 1 = \frac{.0223 \times 82}{1-e^{-(.0223 \times 100)}} = \frac{1.8286}{1-e^{-2.23}} = \frac{1.8286}{.8925} = 2.05 = r_{o,\ 1}$$

$$\text{Tr2} = \frac{.0225 \times 266}{1-e^{-(.0225 \times 100)}} = \frac{5.9850}{1-e^{-2.25}} = \frac{5.9850}{.8946} = 6.69 = r_{o,2}$$

Determination of radium in pc/g ash weight where:

$$\text{pc/g} = \frac{r_o \times 1000}{2.22 \text{ cpm/pc} \times C \times B}$$ where C = 0.5, geometry of proportional counter

and B = 1.02, alpha backscatter

Tr 1 = 2.05 x 1000/1.13 = 1814.16 pc/g ash weight

Tr 2 = 6.69 x 1000/1.13 = 5920.35 pc/g ash weight

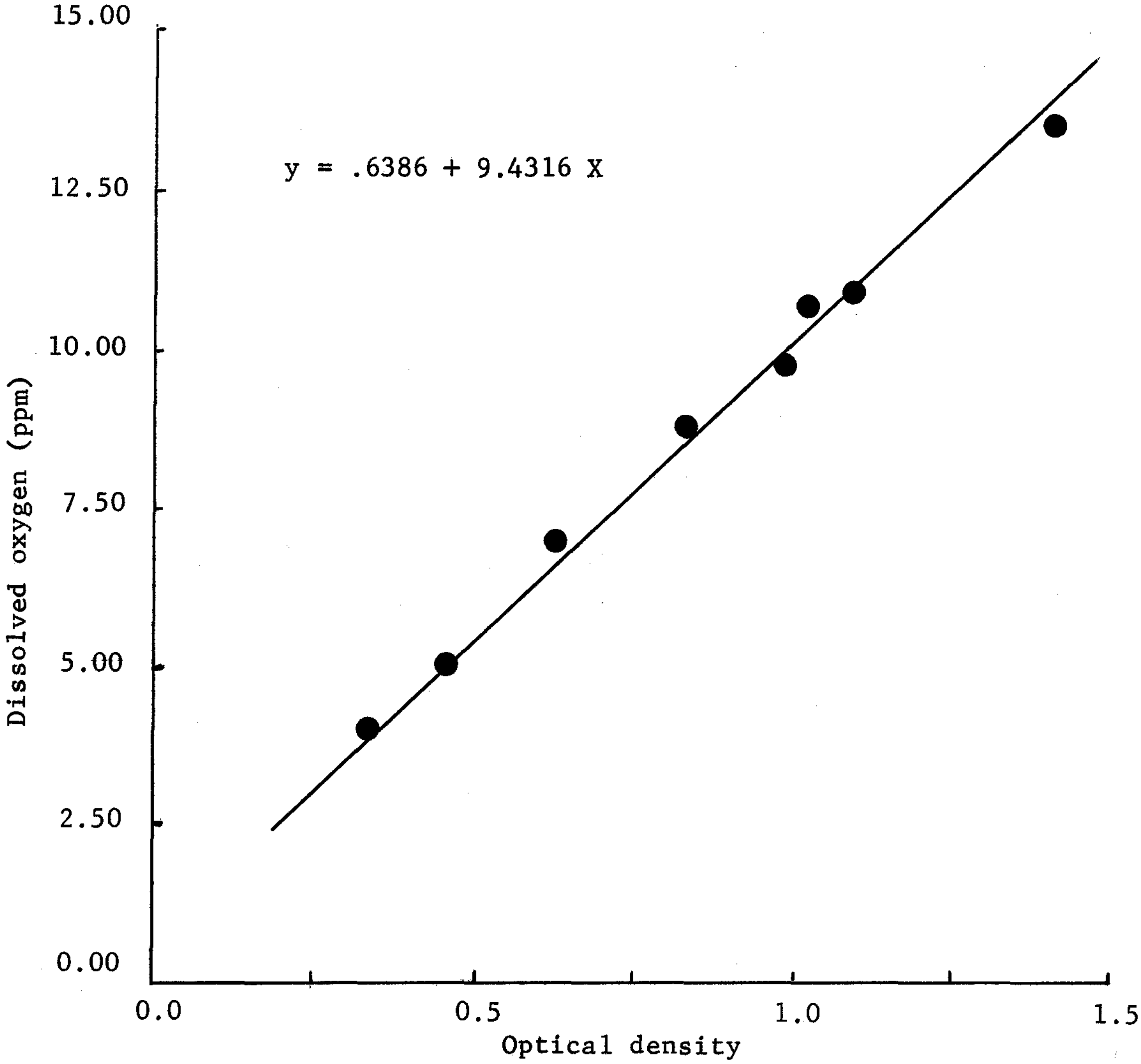

Figure 31. Relationship of optical density to dissolved oxygen content of water using the Winkler technique

Table 51. Three experiments[a] to determine the accuracy of using aliquots of Chlorella culture as an estimate of dry weight. Values are expressed as dry weight in grams per 5 ml aliquot

	Experiment number		
	1	2	3[b]
	.0030	.0227	.0134
	.0026	.0225	.0132
	.0026	.0219	.0129
	.0025	.0233	.0124
	.0026	.0201	.0132
			.0130
			.0123
			.0130
			.0130
			.0131
			.0124
			.0111
			.0125
			.0126
			.0133
Mean	.0026	.0221	.0128
Coefficient of variation	8.1%	5.4%	4.5%

[a]Chlorella cultures were concentrated to different degrees.

[b]Experiment three was the culture used for the start of the major Chlorella experiment.

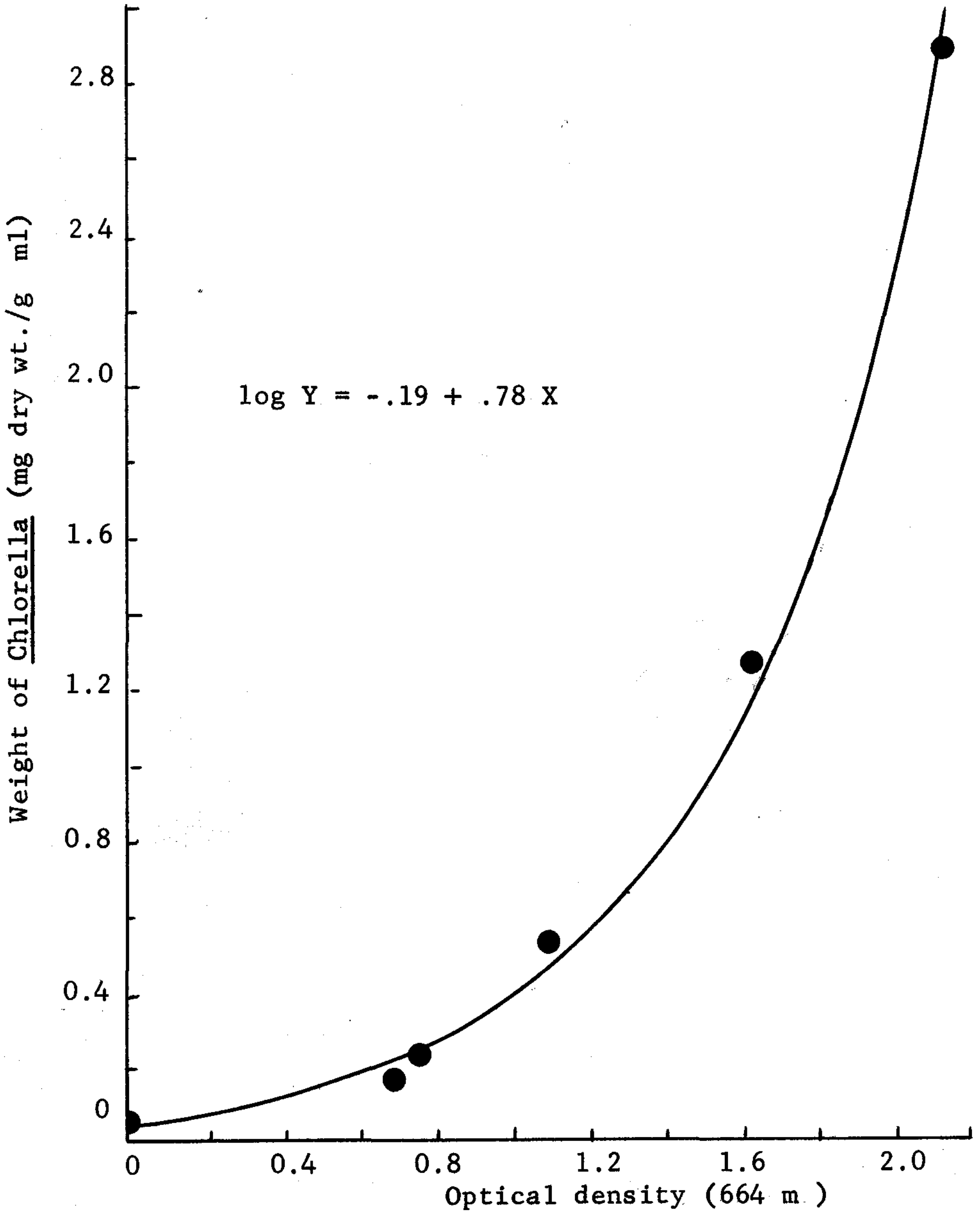

Figure 32. Relationship of the dry weight of Chorella culture to its optical density